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**Amitava Rakshit**, Ph.D (IIT-Kharagpur)

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## EDITORIAL

Food and agriculture position today at a junction. The three challenges – feeding a mounting population, providing a livelihood for farmers, and shielding the environment – must be tackled together if one need to make sustainable progress in any of them. But making headway on this triple challenge is always a difficult job, as creativities in one sphere can have unintended penalties in another. Nevertheless, advancement has often come with social and ecological costs, including water insufficiency, soil degradation, ecosystem stress, biodiversity damage, declining fish stocks and forest cover, and high intensities of greenhouse gas releases. The productive prospective of our natural assets base has been dented in numerous places round the globe, negotiating the forthcoming luxuriance of the planet. Looking forward, the track to comprehensive prosperity is evidently manifested by the 2030 Agenda for Sustainable Development. Disabling the multifarious challenges that the world faces necessitates transformative action, approving the philosophies of sustainability and attempting the core grounds of poverty and hunger to leave no one behind. This special issue attempted to address themes like sustainable transformation in agriculture and food production system, recent advances in aquaculture for food and nutritional security, innovation in global & regional agricultural education towards youth empowerment, climate change resilient agriculture, post-harvest technology for responsible consumption, global and regional policy transformation in greater detail.

Sincerely



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# Role of Multispectral Vegetation Indices in Precision Agriculture – A Review

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## ABSTRACT

Globally, the rising population and exhausting natural resources necessitate the monitoring and management of resources in the best possible way. Precision agriculture deals with the precise supply of agricultural inputs according to the actual crop requirements, thus making the most out of the agricultural practice. The traditional method of field scouting is labor-intensive and time-consuming. The ability to automate the collection, processing and implementation of data increased with new technology viz. GIS, GPS, and remote sensing. Remote sensing can act as a rapid and precise information-gathering technique on spatial variability within the field and temporal variability along long time series. Multispectral vegetation indices were developed to characterize vegetation based on its canopy reflectance. Hence, crop monitoring can be done by using vegetation indices as a surrogate for agronomic parameters. Inferences from crop monitoring based on the vegetation indices can be used for precision agricultural practices and thus achieve better agricultural management. The remote sensing technique with its rapidity and repeatability has got huge potential in analyzing the conditions of soil and vegetation, ultimately leading to applications in sustainable crop and soil management. The paper explores the role of remotely sensed vegetation indices in precision agriculture.

## HIGHLIGHTS

- Concepts of precision agriculture.
- Basics of Remote Sensing.
- Major Vegetation indices and their application.
- Scope and limitations of using multispectral vegetation indices in precision agriculture.

**Keywords:** Vegetation Indices, Remote Sensing, Precision agriculture, Spatial variability, Crop management

With the increasing food demands of the ever-growing world population and depleting natural resources, viz. water, the supply, and quality of water became a major concern which is even capable of challenging food security. The situation gains huge gravity in arid and semi-arid regions which were water-sensitive already. Besides, the agriculture sector has now been transformed into a whole new industry which is even regarded as the silver line of the economy. This changed the outlook on agriculture and necessitated the need for adopting cultivation practices that ensures

better yield and economic returns. Like any other industry, the commercialization of agriculture arises the need for minimizing the inputs and maximizing the profit through maximizing the crop yield. With new-age technologies, farmers get various options to maximize their profit. Market-oriented cultivation practices by anticipating or delaying cropping,

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selling carbon credits to industry, and precision agriculture are a few examples.

Depleting water resources along with increased environmental consciousness also accelerated the shift to precision agriculture. Initiatives by several governmental and non-governmental organizations to increase awareness of achieving a greener safer environment is also notable in stressing the need to lessen chemical fertilizers and pesticides in agriculture.

Variability in crop yield is due to the spatial and temporal variability of several factors viz. soil fertility, landscape, climate, etc. Hence, site-specific management is essential for sustainable agricultural production. The application of remote sensing technology in agriculture helps to identify the spatial variability within a field and temporal variability along a long time series quickly and reliably. The paper reviews the context of using remotely sensed vegetation indices in precision agriculture.

### **Precision Agriculture**

Precision agriculture involves using the information on site variability to effectively manage the sites. It's a combination of several tools viz. GPS, GIS, RS, VRT, and sensor-controlled automatization. It facilitates awareness of the site-specific, practical, and profitable management practices among the agricultural input suppliers, growers, and processors (Lowenberg-DeBoer *et al.* 2002).

The relationship between climatic factors and crop production can be of first-order level (Huggin and Alderfer 1995). Hence, precision agriculture mainly focuses on management based on spatial variability in a standard to fine climatic conditions.

Conventional farming is based on the general assumption that fields are homogenous and hence, treated monotonously. More information on the spatial variability enables site-specific treatment. Thus precision agriculture can effectively reduce the impacts of agricultural inputs such as chemical fertilizers and pesticides on the environment along with increasing crop production.

There would be no need for precision agriculture if the fields were uniform due to the high capital cost involved in it. Usually, large agricultural fields are more observed with spatial variability both

in soil properties and crop productivity owing to their complex arrangement of landscapes and soils. This opens up wide scope for precision agriculture (Tanriverdi 2006).

Precision agriculture renders information for better decision making in agricultural management, provides more accurate farm records, reduces input cost via increased application efficiency, improves crop yield, increases profit margin, and reduces pollution, thereby, promoting improved management of agriculture leading to better yield and better economic returns (Arnold *et al.* 1999).

Concerning irrigation water management, variability within the field can be described by defining zones of difference in total plant available water (TPAW). For higher TPAW gradient over a short distance needs the collection of more soil samples. Though sampling requirements in comparison to a uniform grid can be reduced by using crop yield patterns to determine soil samples, practically, differences in crop yield patterns may not be directly influenced by TPAW variability (Post *et al.* 1988; Tanriverdi 2006).

A major reason for the difficulty in precision agriculture is the time consumed during conventional data collection methods, especially in large field areas. Besides, it cannot be carried out simultaneously. Limitations to precision agricultural practices can be overcome with the combined application of advanced techniques viz. GIS, GPS, and RS.

For precision agriculture, sensing systems for different factors viz. soil, crop, disease, and environmental factors are jointly used with a positioning system (Stafford *et al.* 1997). A large quantity of data can be analyzed at a faster rate and with more accuracy using remote sensing and GIS. Furthermore, it helps in the frequent monitoring of crops. GIS maps can be created using remote sensing and GPS which can be utilized for better resource management. Yield mapping refers to the graphical representation of the quantity of the crop yield on a space and time scale in a map outline using appropriate sensors, GPS hardware, and GIS software. Yield maps help farmers to identify locations within fields where the yield can be improved or inputs can be adjusted to maximize profit along with environmental quality. As the



recommendations for input and farm profitability depend on crop yield, yield mapping has got a huge role in precision agriculture.

### GIS:

Often regarded as the brain of precision agriculture, GIS creates a georeferenced map, and data on position and attributes are stored. A high volume of data can be processed within a short period using GIS. Furthermore, GIS acts as a decision management tool combining data with agricultural knowledge and producing spatially variable recommendations on agricultural inputs. Thus, users can efficiently utilize their resources.

Raster GIS characterizes the spatial variability of a field by partitioning the whole area into regular grid cells while vector GIS makes use of points, lines, and polygons. Statistical analysis is used by many researchers to better identify and represent the relationship between agricultural production and spatial factors (Mulla and Schepers 1997).

GIS-derived spatial variability maps have got applications in guiding agricultural inputs viz. fertilizer, pesticides, water, etc. by analyzing corresponding factors viz. soil organic carbon content, bulk density, soil profile water retention, etc. Besides, GIS maps can be used to assess environmental health viz. ambient air pollution study (Sahu *et al.* 2019).

### GPS

Global Positioning System (GPS) is a satellite-based free navigation system that is developed and maintained by the US. Radio signals from satellites are collected by the receivers which are on the ground and then it is converted to the position data (Bernhardsen 1992). Position data covers all the three dimensions viz. latitude, longitude, and elevation. Hence, GPS helps in obtaining location-specific values for any parameter. GPS supports GIS by geo-referencing both input data and recommendations.

GPS performance has two modes. A single receiver mode collects data on timing which is then converted to position while a differential mode has got two receivers, one stationary and the other on the mobile GPS instrument. Accuracy of positions can be improved up to the range of 1 m using

Differential Global Positioning System (DGPS) receivers. Also, it updates its working conditions to the users.

GPS can be used to precisely study the spatial variability of soil and crop and thus monitor the micro and macro scales of spatial variability in agricultural conditions. Besides, it can generate the architecture of agricultural land viz. field boundaries, field acreage, the disease-affected region, irrigation systems, roads, etc (Sahu *et al.* 2019).

### Remote Sensing

Remote Sensing acts as a spatial information source. Remote Sensing is the method to collect information on objects without being in physical contact with them (Diker 1998; Seyhan 2004). Data is collected by measuring the reflected or emitted electromagnetic radiation from the land surface since it is strongly linked to the surface parameters viz. soil moisture content and vegetation. Thus, these parameters can be derived from reflectance measurements. Reflectance is the ratio of the amount of light reflected from the plane to the irradiance to that plane (Suits 1983).

Components of a remote sensing system are carrier platform, remote sensors, control, and positioning systems, data transmission system, and data preprocessing system that is included under the three broad classes viz. ground base system, spatial foundation system, and remote sensing data storage system (Yin *et al.* 2019). Instruments used for collecting data in remote sensing are called remote sensors and it is usually placed on aircraft and satellites (Diker 1998). Remote sensors are broadly classified as imaging and non-imaging. Imaging instruments gives two-dimensional image of features under observation while non-imaging instruments viz. spectroradiometers measure radiation intensity across a wavelength range of very narrow wavebands. Spectroradiometers can finely differentiate characteristics leading to better interpretation of remotely sensed images and selection of spectral bands for prospect remote sensing imaging devices. These instruments can be used to measure reflected and emitted radiation at immensely small intervals of wavelength with high spectral and temporal resolution. It can be utilized to collect ground truth data (Neale 1983).



Remote sensing using a satellite platform provides consistent, comparable, and cost-effective data of high spatial resolution even from long time series (Foley *et al.* 1998). Free access to multispectral and visible data is an added advantage of certain satellite platforms. Satellite remote sensing allows the collection of data on large agricultural fields at frequent intervals. It can be used to monitor agricultural and hydrological conditions of any land surface. The number of bands obtained by remote sensing is increasing as high-resolution spectral instrumentation is used, and the bandwidth is narrowing (Honkavaara *et al.* 2013).

The major advantage of remote sensing over conventional mapping techniques is that it can derive complex relationships even from an aerial view (Grenzdorffer 1997). Remotely sensed data has got direct and indirect applications. Sensor captured images can be directly used for any purpose or information is derived from the image and then this information is used for any management purpose (Frazier *et al.* 1997). Remotely sensed data from terrestrial vegetation have got applications in the broader fields of agriculture, biodiversity conservation, environmental monitoring, forestry, urban green infrastructures, etc.

### Remote sensing in precision agriculture

Conventional methods of taking ground measurements of various vegetation parameters viz. leaf area, biomass, etc. are expensive and tiring. Remote sensing methods utilize the spectral reflectance of vegetation and soil to obtain their attributes. It is based on the fact that any surface reflects, absorbs, or transmits incident light. The length of the growing season was found to be irrelevant since the fresh and green leaves accounted for similar reflectance spectra (Sinclair *et al.* 1971; Neale 1983).

Remote sensing of vegetation is majorly based on the ultraviolet (10 to 380 nm), visible (450-750 nm), and the near and mid-infrared (850-1700 nm) regions of light spectra (Rahim *et al.* 2016; Cruden *et al.* 2012). The wavelength spectrum responded differently to leaf characteristics— visible light was absorbed by pigments, the NIR region was affected by internal leaf structure and the wavelength spectrum beyond that was influenced by the concentration of water

in the tissue and to some extent, affected by leaf structure (Tanriverdi 2006).

Reflectance spectra obtained from plants vary with plant variety, growth stage, the moisture content in tissue, and other intrinsic characteristics (Chang *et al.* 2016). Morphological and chemical traits of the surface of leaves or organs are another determinant factor of spectral reflectance (Zhang and Kovacs 2012).

Reflectance measurements can be used for determining chlorophyll content in leaves which can be related to the nitrogen grade of the vegetation (Thomas and Oerther 1972). Spectral reflectance from plants in the thermal infrared region (8000-14000 nm) can be directly linked to its temperature and hence, can be used for stomata dynamics assessment (Xue and Su 2017). The variances and changes in the green leaves of plants, as well as canopy spectral properties from remotely sensed photos, are used to analyze vegetation information.

Data required in precision agriculture, for site-specific management can be categorized into three classes. Conditions that are constant throughout the season viz. soil properties are classified as information on seasonally stable conditions and hence, it has to be measured only at the beginning of the season whereas information on seasonally variable conditions is the class of dynamic conditions throughout the season viz. moisture content of the soil, diseases and pest infestation of crop and therefore, it has to be monitored throughout the cropping season. The third class is the information to identify the factors for yield spatial variability and to develop a management strategy, which determines the causes of the variability as it is comprehensive of the first two categories. Remote sensing can be used to acquire all the three categories of information for the successful implementation of precision agriculture (Moran *et al.* 1997).

Remote sensing has got applications in terms of both macro and micromanagement in the field of agriculture such as vegetative cover estimation, canopy temperature monitoring, stress identification, water deficit determination, irrigation scheduling along with yield prediction (Mulla 2013).

Direct application of remote imagery gained popularity with the advances in technology like GPS and sensors leading to better affordability





and availability. The real-time position can be superimposed on the remotely sensed imagery using GPS and any point within the photograph can be accessed. And thus, variability in soil and crop can be analyzed and managed directly from images.

Another major direct application of remote sensing in precision agriculture is the extraction of management zones from remote sensing imagery-derived vegetation indices maps using GIS software. When integrated with variable rate sprayer equipment, site-specific requirements are met with the help of real-time sensors, leading to reduced groundwater contamination and improved nutrient use efficiency (Schepers and Francis 1998).

The indirect application includes the preparation of a base map with different layers of information using GIS, soil mapping across the field, use of remotely sensed vegetation parameters in crop simulation models, identifying location and factors causing crop stresses viz. diseases, insects, and weeds, improving soil sampling strategies with remotely measured soil and plant parameters. Soil salinity areas can be mapped using remote sensing data (Basso *et al.* 2004).

## Vegetation Indices

Vegetation indices are in general, either ratios or linear combinations of spectral reflectance corresponding to different wavelengths obtained from radiometer bands. They can give better correlations with vegetation parameters viz. plant height, green leaf area index, leaf chlorosis fraction, the water content in leaves, vegetation cover percent, and wet and dry biomass, than the individual bands. (Bausch and Neale 1987). Hence, vegetation indices were introduced to monitor and analyze these agronomic parameters.

Vegetation cover can be estimated from vegetation indices derived from the reflectance of the canopy. This estimated vegetation cover can be used instead of measured vegetation cover (Moran *et al.* 1994). Thus, crop monitoring can be done which can be used for improved management through precision agriculture. The water deficit of any plant at any growth stage can be estimated from crop monitoring based on reflectance measurements. Thus, water management can be done which is the major part of agricultural crop management.

Vegetation indices derived from remote sensing are the most effective but simple algorithms to quantitatively and qualitatively analyze vegetation cover along with vigor and its growth dynamics. There is no single mathematical expression for defining every vegetation indices. Since each of them is tailored to suit specific applications and is derived from visible and non-visible spectra, their applicability depends on the platforms and instruments involved (Xue and Su 2017). Indices developed from near and mid-infrared regions can represent various characteristics of plants viz. pigments, water content, sugar content, carbohydrate content, protein content, aromatics, etc. apart from proxy quantification of growth and vigor (Foley *et al.* 1998). Multispectral vegetation indices derived from the reflectance of the canopy in comparatively broader wavebands can be used to monitor the vegetative growth corresponding to climatic variables (Hatfield and Pinter 1993).

Ratio Vegetation Index (Jordan 1969) is a simple ratio index with high sensitivity to vegetation and a strong link to plant biomass. Hence, RVI is commonly used for the estimation and monitoring of green biomass, especially when vegetation coverage is very dense. In sparse vegetation conditions, they are susceptible to atmospheric effects leading to poor representation of biomass. Difference Vegetation Index or the Environmental Vegetation Index (Richardson and Weigand 1977) are used to monitor the ecological environment of vegetation and is highly sensitive to soil background changes.

Vegetative growth at any stage can be monitored using the Normalized Difference Vegetation Index (Rouse *et al.* 1973) and Perpendicular Vegetation Index (Richardson and Wiegand 1977). Besides, PVI can efficiently eliminate soil background effects. Major applications of PVI are LAI calculation, surface vegetation parameter (grass yield, chlorophyll content) inversion along with crop identification and classification (Kaufman and Tanré 1992; Wenlong 2009). Adjustments have to be done to combat sensitivity to soil brightness and reflectivity.

The most widely used vegetation index is the NDVI, even used in local and global assessments of vegetation and is highly sensitive to green vegetation. Furthermore, NDVI requires remote sensing



calibration due to its sensitivity to atmosphere, cloud and cloud overcast, leaf canopy brightness and shadow, soil brightness, and soil color. NDVI is correlated to canopy photosynthesis along with canopy structure and LAI. Many researchers have correlated NDVI with Leaf Area Index (Xue and Su 2017).

Several modifications were done on NDVI in an attempt to increase the ability to derive better interpretations. Soil Adjusted Vegetation Index (Huete 1988) and Modified Soil Adjusted Vegetation Index (Qi *et al.* 1994) minimizing the effect of soil background are examples. Concerning the low noise level, SAVI was suggested as a better vegetation indicator among other basic vegetation indices. In an attempt to optimize the L factor in SAVI, the MSAVI was developed with a variable correction factor, L. This L varies with canopy cover and soil type. A Modified Normalized Vegetation Index (Liu and Huete 1995) integrates both atmospheric and soil adjustment factors.

Stress related Vegetation Index (Gardener 1983) and Cubed Ratio Index (Thenkabail *et al.* 1994) were developed from the Mid-InfraRed Band. Visible Atmospherically Resistant Index (VARIgreen) is another example of vegetation index being in a strong relationship with measured vegetation cover (Gitelson *et al.* 2001).

Though few researchers found that measured vegetation cover represents the vegetation cover in a better way than the estimated vegetation indices, the practical difficulties involved in a measuring method viz. time required to complete a data set, possible crop damage, usage of instruments for the entire growing season has to be considered. Hence, using vegetation indices over measured vegetation cover has got advantages like lesser time, lesser cost, minimum labor requirements, and large data availability, especially in larger areas (Tanriverdi 2003).

### Scope and Limitations of Vegetation Indices in Precision Agriculture

As vegetation indices are developed from single or limited bands, lack of sensitivity in acquiring data, especially from heterogeneous canopies viz. horticultural tree plantations, might be counted as a shortcoming. Extraction of vegetation indices

from regions of interest becomes difficult in such areas due to the variations in soils, weeds, cover crops, and vegetation of interest, especially when a single plant has got different vegetation indices because of spatial variability. Image processing steps viz. filtering and denoising get complicated if the vegetation indices of the study crop are similar to that of other crops. However, a simplistic vegetation index algorithm can give simple but effective tools to quantify the vegetation status on the land surface (Hoffmann *et al.* 2015; Xue and Su 2017).

The spectral resolution of remotely sensed data used for the extraction of vegetation indices is yet another challenge to be addressed. Though vegetation indices developed from broader wavebands can identify various stress, they cannot recognize the exact factors causing a specific type of stress. Hence, physiological stresses viz. water and nutrient shortage are better correlated with narrow-band indices like Water Band Index, Normalized Pigment Chlorophyll Ratio Index, Canopy Chlorophyll Content Index, and Photochemical Reflectance Index (Basso *et al.* 2004).

Spatial resolution and orbit period of satellite platforms are the two major concerns in the precision agriculture applications viz. nutrient and water management. Also, the use of a large number of sensors can make the whole process expensive for long time series. The unavailability of satellite data on cloudy days due to the inability of passive sensors to penetrate through clouds is another concern. Though this issue can be partially rectified by the usage of airborne platforms and Unmanned Aerial Vehicle platforms, it has got its share of drawbacks. The airborne remote sensing method requires air crafts and pilots and hence can be expensive. However, Unmanned Aerial Vehicle platforms have got high temporal and spatial resolution (sub-meter resolution). It makes use of affordable aircraft and camera payloads from visible to thermal infrared and also, 3D LIDAR, and thus, a better alternative platform for assessing plant growth and vigor, irrigation scheduling, evapotranspiration modeling, etc (Xue and Su 2017).

Less developed countries don't much prefer precision agriculture tools since their smaller agricultural areas and their corresponding cost of agricultural inputs doesn't signify the use of precision agriculture. The initial cost involved in



using precision agriculture techniques is more in small field areas. Hence, conventional farming techniques are found to be more affordable than precision agriculture techniques in improving the management of agriculture. However, the scattered nature of these agricultural fields necessitates field use planning. Furthermore, precision agriculture offers better results in terms of time, labor, and data availability. It would be better to determine the field size for which precision agriculture is significant considering factors such as water supply and cost of precision agriculture (Tanriverdi 2006).

Technological illiteracy among the farmer community might give rise to the hesitation in adopting advanced technology like precision agriculture. Inadequate knowledge and technical expertise in analysis and decision making using a computer at both local and regional levels are probably the major constraints in the success of precision agriculture (Sahu *et al.* 2019).

Requirements for successfully using remotely sensed data in precision agriculture are the high spatial resolution (preferably, not more than 10 m), frequent coverage, timely availability, low cost, and integration with expert systems (Tanriverdi 2003; Sahu *et al.* 2019). Precision agriculture tools (GIS, GPS, and remote sensing) together can provide a complete data set that is accurate to use in precision agriculture, especially in heterogeneous or large agricultural areas. For the best outcome from precision agriculture, potential limiting factors to crop profitability must be identified and addressed. Poor aeration, Soil water availability, and weed pressure are a few examples of limiting factors. A better understanding of spatial variability in yield and factors responsible for it, both environmental and managerial, will give a finer idea of best-suited site-specific agricultural operations. With the better correlations evolved between these factors and yield, improved spatially variable recommendations can be obtained (Tanriverdi 2006). Integrating remotely sensed data with crop simulation models has a huge prospect in agriculture applications (Basso *et al.* 2004). Rapid mapping of insect infestation, treatment logging by variable-rate spray operators, and pattern study of disease spread are a few of the aspects which can be incorporated into precision agriculture with the help of remote sensing (Sahu *et al.* 2019).

## CONCLUSION

Remote sensing acts as a spatial information source based on the spectral reflectance from vegetation and soil. It has got direct and indirect applications in precision agriculture. The use of vegetation indices to quantify spatial variability within the field and thus manage crops and resources is one such application. Vegetation indices are extracted from the reflectance data obtained from the remotely sensed images. Generally, Vegetation indices are developed from the reflectance measurements from NIR and red bands, combined in various ways. The major advantage of remote sensing in agriculture is its non-destructive nature along with its accuracy and swiftness. Near real-time data obtained from remote sensing helps in actively managing the inputs and thus maximizing yield and economic returns. Vegetation indices derived from remotely sensed data can identify crop stress conditions to a large extent. As each vegetation index was developed to address a different concern, judicious use of vegetation indices is necessary to point out the crop conditions along with underlying factors. High cost in image acquisition, coarse spatial resolution, and insufficient temporal and spectral resolution are the major limiting factors of the use of satellite images in precision agriculture. Major limitations can be overcome by increasing the spatial, spectral, and temporal resolution of remotely sensed data. Free access to high-resolution data is now made available by various agencies like USGS earth explorer while freeware like QGIS is available for the processing of satellite data. As the technology develops, the availability, reliability, resolution, and cost-effectiveness of the remote sensing images are expected to improve further and will lead to better interpretation of vegetation indices on the spatial and temporal variability of crop and soil. The importance of providing accurate and relevant information at a faster pace and low cost is needless to emphasize, for the effective use of remotely sensed vegetation indices in precision agriculture. Thus, just like in every other sector, remote sensing applications become the need of an hour in the agriculture sector.

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# Three-Dimensional Printing in Food Process Engineering: A Prospective and Retrospective Analysis

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## ABSTRACT

Three-dimensional printing technology is a novelty which is going to usher the new aeon of localised manufacturing by promising to transfigure food formulation as well as manufacturing process. As compared to the food manufacturing processes based on robotics, three-dimensional food printing combines both the 3D printing and the technique of digital gastronomy to fabricate food products with personalization in shape, texture, color, even nutrition and flavor. This brings up artistic proficiencies to fine dining and extend personalization capabilities to industrial culinary sector. 3D printing is a technology which uses a number of additive manufacturing techniques for food product fabrication. The objective of the study is to analyse various printable materials, printing platforms, and the food printing mechanisms. This study also provides a better insight towards the different advantages and limitations of food product fabrication using 3D food printing. This study is done to analyse and summarise published papers and articles relating to 3D printing, its impact on food process engineering and also to provide an insight to the direction of its future. The printing technique comprising of extrusion based and inkjet printing and binder jetting. From this technology review, it's clear that the food printing may exert a significant influence on various types of food processing, which allow designers/users to manipulate/customize forms and materials with enhanced and unprecedented capability.

## HIGHLIGHTS

- ① Different materials that can be used for 3D food printing were discussed.
- ① Technologies adopted for printing were reviewed.
- ① Advantages and limitations of food customization were analysed.

**Keywords:** 3D food printing, printing platforms, multi-materials, extrusion-based printing, sintering technology

Food, consisting essentially of protein, carbohydrate, fat and other nutrients used in the body of an organism, being any substance that is eaten, drunk or taken into the body to sustain life. Food products are rapidly evolving, so there is an increasing demand for its manufacturing field to follow up the novel practices and tools to work effectively. About 15-25% of people over 50 years of age endure from swallowing difficulties. Pureed foods not only overcome this difficulty but also provides the necessary nutrition to them, but most of them are unpleasing and unappetizing. Customization has been marked as the driving

force to hamper conventional ways to produce and deliver food. There is an expanding market demand for mass customization on colors, textures, shapes, nutrition flavors etc., which covers many of the food products including customized ice creams, cakes, coffees, hamburgers and biscuits. Most of the food structuring techniques are developed for mass production. Personalized foods are

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generally designed by specially trained artisans using methods, which involves assembling of prefabricated parts to meet customer demands. But producing a small number of such pieces are not at all economical (Sun *et al.* 2015a). So, in order to achieve mass customization economically, an innovative technology to design and fabricate food is necessary.

Three-dimensional food printing, established since 1980's, alias additive layer manufacturing, is a novel technology which unites additive manufacturing, numerical control technology, computer technology, material science technology and precision drive technology to produce custom designed 3D food objects in a layer-by-layer fashion without object specific moulding, tooling or human intervention (Yang *et al.* 2019). Thus, this technique can enhance production efficiency and decrease manufacturing cost for personalized food product fabrication. Also, it has its advantages on food structure personalization, broadening food raw material source, shortening of food supply chain and customized nutrition (Godoi *et al.* 2016; Chen 2016).

This food printing, an automated manufacturing technique, has achieved increased attention for its unique ability to make complex geometric structures, allowing mass production with environment as well as economic benefits, leans on layer-by-layer deposition (Perez *et al.* 2019). The object designs are made by Computer Aided Design (CAD) software and the food printer is generally connected to a computer system through a USB cable. The products are fabricated on the basis of 3D models and can be structured into any shape in theory. 3D printers are robotics-based machines that can be used fabricate objects through additive manufacturing. In this, printers take only the essential matter in powder or filament, and liquid forms, which the printer then melts or solidifies to obtain the objects final shape, instead of defining shape by eliminating excess raw materials. This process holds great scope for manufacturing as it exceptionally amends all the supply chain dynamics, eliminate the need for economies of scale and also reduces cost, energy consumption, time, and transportation requirements.

In food processing industry, the 3D printing technique satisfies the need of consumers in accordance with their occupation, age, gender

and lifestyle by flawlessly integrating nutrition and enables manufacturing of customized foods (Rodgers 2016). Usually dehydrated raw materials are preferred for 3D printing to evade the deterioration of raw/fresh ingredients (Nachal *et al.* 2019). The 3D printers have an ability to use dehydrated foods or can dehydrate the foods after printing. This makes 3D printing technology convenient for producing space foods as compared to the traditional processing methods where it requires longer nutritional stability (Terfansky and Thangavelu 2013). By altering or eliminating or decreasing the protein, fibre, vitamin, cellulose and fat contents, and by introducing some functional compounds including carotenoids and anthocyanins to give desired functions, with relative ease, 3D printing technology can produce personalized food for pregnant women and athletes (Dankar *et al.* 2018). These printed foods with upgraded taste and visual appeal are suitable not only to elderly people and patients with paralysis, Parkinson's disease and stroke but also for children and teenagers with specific nutritional needs (Portanguen *et al.* 2019; Hamilton *et al.* 2018). In fact, 3D printing facilitates fabrication of customized foods, having both health promoting as well as enjoyment factors (Severini and Derossi 2018).

This study systematically reviews about the 3D printing technology, in detail, applied to food process engineering. The review provides insights about the available printing materials, functional ingredients used in printable food formulas, food printing platforms, printing recipes and various 3D printing technologies. Also, it explores the relative advantages of this technique in maintaining quality of 3D printed foods and features prospects of future research.

## AVAILABLE PRINTING MATERIALS

The available materials for food printing are categorised in to three; natively printable materials, non- printable traditional food materials and alternative ingredients (Sun *et al.* 2015a).

### Natively printable materials

Natively printable materials are the materials that can be smoothly extruded from a syringe and which shows the ability to maintain their shape after deposition and no further post processing



is required after printing. These materials include hummus, cake frosting, hydrogels, chocolate and soft cheese (Dankar *et al.* 2018). The highlight of such materials is that the food products made by them can be personalized fully for nutritional value, taste and texture. Although, majority of them are not treated as main courses so these are generally reserved for space as well as medical applications. Certain formulations including protein pastes doesn't retain their shape easily so it requires further processing to improve nutrition absorption and taste (Cohen *et al.* 2009; Lipton *et al.* 2010; Sun *et al.* 2015b).

### Non printable traditional materials

The foods which are consumed by people on daily basis such as rice, fruits and vegetables, meat etc. are not generally printable in nature. In order to make them capable for extrusion, additives (hydrocolloids) can be added and also it can be utilized by many culinary fields. Using gastronomic tricks, certain semi solid and solid food items have been already altered to become printable but when it comes to the entire list, it's really difficult to modify and test. Fabricating an element set by using a limited number of ingredients assuring high degree of freedom on flavor and texture is the only potential solution. Concentration of xanthun gum, gelatin and certain other hydrocolloids are fine tuned to obtain a great range of textures (Cohen *et al.* 2009). A greater number of traditional edibles requires post deposition processing (baking or steaming) after printing, but it finally result to a non-homogenous texture. So, in order to modify cooking recipes for printing as well as post deposition cooking, Lipton

*et al.* (2010) conducted an investigation and found out one particular recipe that can print complex 3D models and maintains their shape even after deep frying.

### Alternative ingredients

These ingredients extracted from fungi, insects, lupine, algae and seaweeds are the emerging sources of fibre and protein. Combination of extrudable icing, soft cheese and insect powders were utilized as printing materials for making tasty pieces and shaping food structures in the 'Insects Au Gratin' project (Southerland *et al.* 2011). Agricultural as well as food processing residues can be used as sources of eco-friendly printing materials, by converting the residues in to enzymes, biologically active metabolites and food flavor compounds. In short, including alternative ingredients to 3D printing would benefit in creating healthier food products (Sun *et al.* 2018).

### 3D FOOD PRINTING TECHNOLOGIES

Food pieces are created in a layer-by-layer fashion using food printing, which doesn't need any high energy source for completely extracting liquid components from food composition. After deposition, the layers need not be fully solidified but the layers should process adequate rigidity and strength to hold up their own and subsequent layer's weight without remarkable deformation or change in shape. The standard of food items, which are fabricated using food printing technology rely on the process and planning instead of people's skill. A comparison between 3D food printing technologies is shown in Table 2.

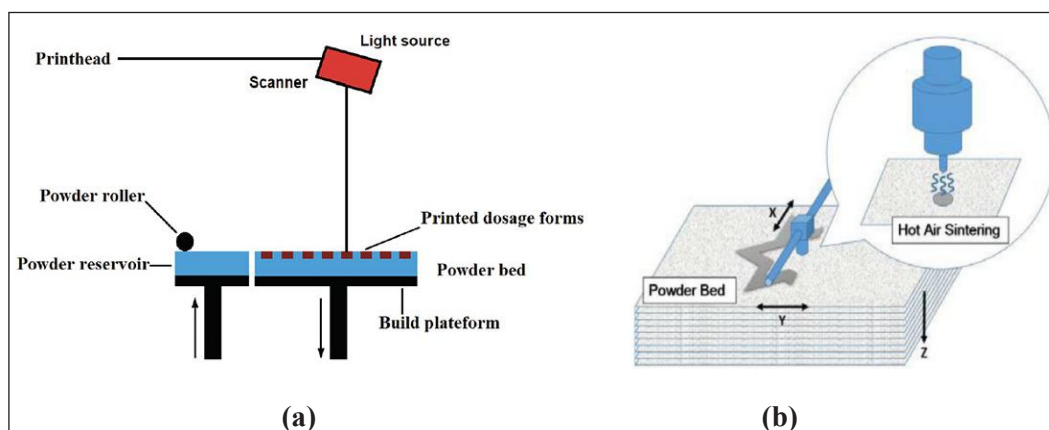


Fig. 1: (a) Selective laser sintering (b) Selective hot air sintering

**Table 1:** Overview of various food materials successfully printed using extrusion-based 3D-printing

Class	Material	Additive	References
Protein	Turkey meat and scallop	Transglutaminase	Lipton <i>et al.</i> 2010
	Cereal dough snack with yellow mealworm powder	Sodium chloride	Wang <i>et al.</i> 2017 Severini <i>et al.</i> 2018
	Fish surimi gel		
Lipids	Bacon fat		Lipton <i>et al.</i> 2010
	Chocolate		Godoi <i>et al.</i> 2016
	Cheese		Le Tohicet <i>et al.</i> 2018
Carbohydrates	Lemon juice gel	Potato starch	Lipton <i>et al.</i> 2010
	Mashed potato	Potato starch	Derossi <i>et al.</i> 2017
	Fruit snack	Pectin	Yang <i>et al.</i> 2018a
	Smoothie	Fish collagen	Severini <i>et al.</i> 2018
	Pectin	Agar, alginate, glycerol, lecithin	Lille <i>et al.</i> 2018
	Hydrocolloids		Chuanxing <i>et al.</i> 2018
	Baking cookies	Pea protein	Kim <i>et al.</i> 2018
	Fruit snack		
	Skim milk powder		

### Selective sintering technology

Selective sintering technology is a technique that generally uses a power laser or hot air as a source to selectively bind the particles in powder form layer by layer and finally to a 3D structure. In order to sinter NesQuik powders and sugars, TNO's food jetting printer used laser and thus built 3D objects (Gray 2010). The material, which is sintered forms the product part while the remaining un-sintered powder provides support to the structure. A low velocity hot air stream is used to melt and sinter a bed of sugar by the CandyFab (2007). The created powder bed is heated to a temperature which is just lower than the melting point of that material so as enable fusion to the preceding layer and to reduce thermal distortion. Both hot air and laser sintering processes enables to rapidly build food items in a short span of time without any further curing. However, their limitation is that they are only appropriate for fat-based materials and sugar, having relatively low melting point, and also as it involves so many variables, machine structure as well as the fabrication processes will be complicated (Sun *et al.* 2018).

### Extrusion based 3D Printing

The process of forcing solid/semi solid/liquid materials via die opening for fabricating objects with required cross-section is extrusion (Sun *et al.* 2018).

Extrusion based food printing techniques depends on continuous flow of ink in layer-by-layer manner, usually heavily concentrated colloidal inks. Based on the ability of a material to form gel or to achieve paste consistency, the total solids can range from 5-50% concentration. When a pressure gradient  $\Delta P$  through a length of  $l$  is applied, the ink starts flowing through the nozzle. Thus, according to Eq. 1, a radially changing shear stress ( $\tau_r$ ) is developed, where  $r$  denotes the radial position within nozzle (Godoi *et al.* 2016). At the centre of nozzle wall ( $r=R$ ), velocity is zero, and at centre ( $r=0$ ), velocity is maximum (Lewis 2002). The absolute aim of this method is to obtain the output of food extrusion cooking with customized nutrition control and digitalized design.

$$\tau_r = \frac{r\Delta P}{2l} \quad \dots(1)$$

Extrusion based food printers are badly limited by material choices, delamination due to temperature fluctuation and long fabrication time but these printers are of compact size and low maintenance cost (Sun *et al.* 2018). Table 1 gives an idea of various food materials that can be printed using extrusion-based 3D Printing.

### Melting Extrusion

Materials in the form of powder, filament and paste

are generally considered as the suitable materials for melting extrusion. In order to ensure printability, it is necessary to control temperature during printing of fat or sugar rich pastes. Chocolate, the most commonly used edible ink because of its melting properties, can be fed to the reservoir of printer either in powder form or in paste like/melted form (Mantilal *et al.* 2020). Edible filaments are rarely produced and printed. Goyanes *et al.* (2015) created various filaments, which contain caffeine or paracetamol in a water-soluble polymer appropriate for 3D printing into different pharmaceutical dosage forms. If the filament production is followed by fused deposition modelling (FDM), then the post processing procedures which are generally required for food printing can be avoided. In food processing applications, it's believed that the food grade filaments will find its usage in the confection of packaging of foods (Godoi *et al.* 2019).

### Hydrogel Forming Extrusion (HFE)

It is the method of extruding hydrocolloid solutions into a gel/polymer setting bath with the help of a vibrating nozzle, syringe pipette, jet cutter and similar apparatus. Diameter of these gel droplets should be in the range of 0.2-5 mm and the key to form stable shapes in HFE is controlling solution's temperature (Sun *et al.* 2018).

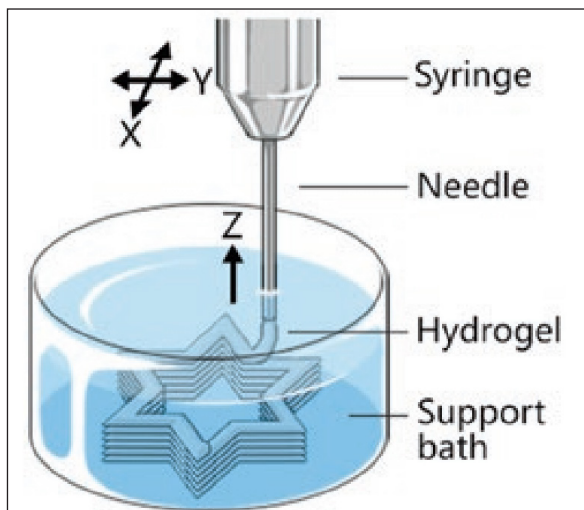


Fig. 2: HFE

This process exclusively depends on the gel forming mechanism and the rheological behaviour of polymer. Serizawa *et al.* (2014) created an edible gel 3D printer using a dispenser and syringe pump for producing soft foods for those who have swallowing

difficulties. This temperature control is utilized for making many economic machines including HFE for fruit printing (Molitch-Hou 2014), hot melt extrusion (HME) for printing chocolate (Choc Edge 2014), room temperature extrusion (RTE) for printing (Alec 2015; Molitch-Hou 2014).

### Binder Jetting

In this technology, every single layer of powder is uniformly distributed across the platform of fabrication, and then a liquid binder sprays to join two consecutive layers (Sachs *et al.* 1992). In order to stabilize powder material and reduce disturbance occurs due to binder dispensing, a layer of water mist should be sprayed before fabrication. Sugar Lab in 2013 utilized different flavor binders and sugar for fabricating sculptural cakes which are complex in nature for special events and the fabrication utilized 3D System's color jet printing technique (3D Systems 2013). Binder jetting provides fast fabrication with low material cost, but at the same time, it suffers from high machine cost and rough surface finish (Sun *et al.* 2015a). Also, in most cases it requires post processing, including curing at increased temperature, for strengthening the bonding.

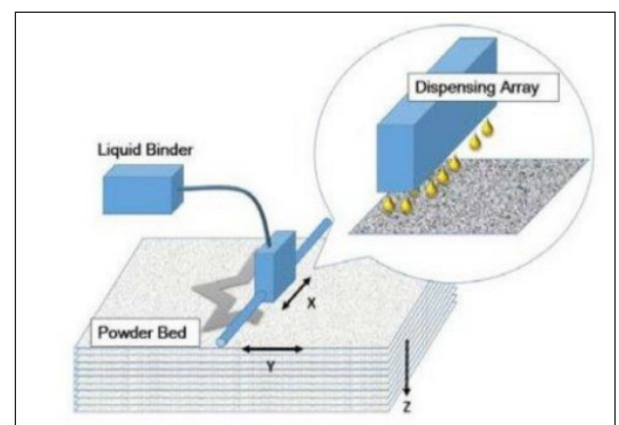


Fig. 3: Powder bed binder jetting

### Inkjet Printing

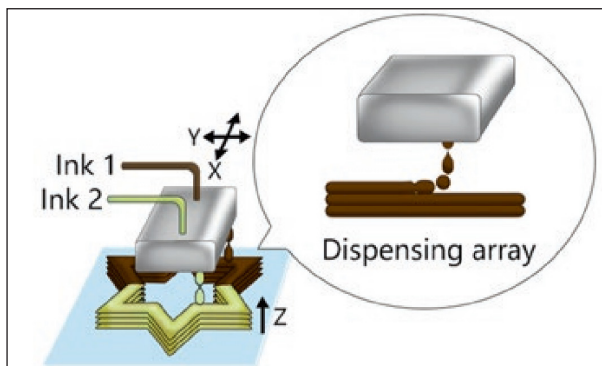
Commercialization of Inkjet printing technology in food printing had done by the company FoodJet (FoodJet 2019). Inkjet printing uses an array of pneumatic membrane nozzle-jets which dispenses a stream of droplets of food ink onto cookies, biscuits, cupcakes, and pizza bases (Kruth *et al.* 2007). Two types of inkjet printing methods are

**Table 2:** Comparison of 3D food printing technologies

	Sintering technology	Melting extrusion	Binder jetting	Inkjet printing
Materials	Low melting powder such as sugar, NesQuik or fat.	Food polymers (chocolate)	Powder such as starch, sugars, flavors, corn flour and liquid binder	Low viscosity materials (paste or puree)
Platform	1. Motorized stage 2. Sintering unit 3. Powder bed	1. Motorized stage 2. Heating unit 3. Extrusion device	1. Motorized stage 2. Powder bed 3. Inkjet print-head for binder jetting	1. Motorized stage 2. Inkjet printhead 3. Thermal control unit
Printing resolution	Powder size: 100 $\mu\text{m}$	Nozzle dia: 0.5-1.5 mm	Nozzle dia $\leq 50 \mu\text{m}$ Powder particle $\geq 100 \mu\text{m}$	Nozzle dia $\leq 50 \mu\text{m}$
Fabricated products	Food-grade art objects, toffee shapes	Customized chocolates	Sugar cube in full color	Customized cookies, Bench-top food paste shaping
Pros	1. Better printing quality 2. Complex design	1. Cost effective 2. Fast fabrication	1. More material choices 2. Better printing quality 3. Full color potential 4. Complex design	Better printing quality
Cons	1. Expensive platform 2. High power consumption 3. Limited materials	Low printing quality	1. Slow fabrication 2. Expensive platform	1. Slow fabrication 2. Expensive print-head 3. Expensive platform 4. Limited materials
Machine	Food jetting printer	Choc Creator	Chefjet	Foodjet
Company	TNO	Choc Edge	3D systems	De Grood Innovations

Sun et al. 2015b; Godoi et al. 2019.

there: Continuous Ink-Jet Printing (C-IJP) and Drop-on-Demand Inkjet Printing (DoD-IJP). In the former, ink is dispensed continuously through a piezoelectric crystal vibrating at a constant frequency. The later, DoD-IJP, ink is ejected out from heads under pressure exerted by the valve. In DoD-IJP the printing rates are slower than that of C-IJP systems, but the precision and resolution of produced images are higher. However, DoD-IJP requires the use of an electrically conducting fluid which limits the application in food customization.



**Fig. 4:** Inkjet printing

In general, inkjet printing deals with low-viscosity

materials which don't have enough mechanical strength to hold a 3D structure. Therefore, it is rather used for printing drawings over flat surfaces as surface filling or Image decorations (Pallottino et al. 2016). Grood and Grood (2011) had created DoD-IJP to lay edible liquid onto food surfaces to create appealing images. The FoodJet printer uses pneumatic membrane nozzle jets to dispense edible drops onto a moving object to form an appealing surface (FoodJet 2019).

### PRINTING QUALITY ASSESSMENT

There are generally two important parameters by which the printing quality of a final 3D product is judged. They are shape fidelity and mechanical properties. The criteria for evaluating the printing quality are explained in Table 3.

#### Shape Fidelity

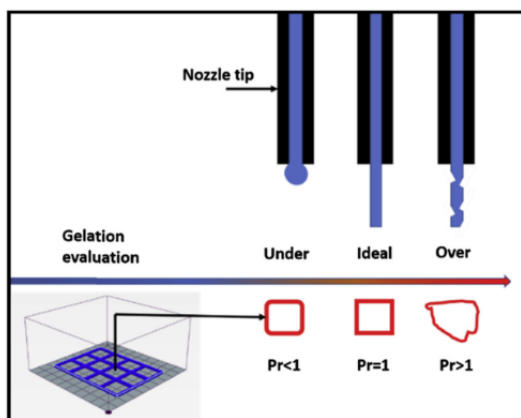
He et al. (2019) illustrated that except from printing parameters including printing distance, air pressure and feed rate, lattice area (A) and line distance (D) will also have an impact on gel lattice's printing quality. The optimum condition for printing can

**Table 3:** Criteria for printing quality evaluation

Printing Quality Score	Description
5	The paste should pass through nozzle smoothly. Shape should be supported with excellent precision. Printed and designed size should have a deviation less than 2%. There should not be any distortion of layer-by-layer structure.
4	The paste should pass through nozzle smoothly. Printed and designed size should have a deviation should be in the range of 2-5%. The layer which is distorted is within 5% of the entire layer-by-layer structure.
3	The paste should pass through nozzle. Printed and designed size should have a deviation should be in the range of 5-15%. The layer which is distorted is within 5-15% of the entire layer-by-layer structure.
2	The paste should pass through nozzle but the shape get collapse while printing or the collapse within 30 minutes after printing the complete shape.
1	Because of low viscosity, the printed shape will not be able to support the shape or the paste could not flow through the nozzle because of high viscosity.

Liu and Ciftci 2020.

also be evaluated by observing the filament material shape extruded from nozzle. The optimum gelation, characterized by an evenly extruded filament in a smooth surface and in three dimensions is illustrated in Fig. 5. It is possible to observe the liquid like behaviour of ink and droplet formation at the end of nozzle at stages of under gelation. A stiff filament with cracks all over its length can be observed when the ink is in over gelation stage. In a lattice structure, Ouyang *et al.* (2016) exemplified that the printability and circularity level of internal square areas can be correlated. The quality of printing of gel-like inks can be evaluated using Eq. 2 and 3. It has been observed that if  $Pr=1$ , ideal gelation is obtained and if  $Pr<1$  or  $Pr>1$ , it is the stage of under gelation and over gelation respectively.



**Fig. 5:** Schematic demonstration of filament quality. At the left end, 3D lattice is shown emphasising the internal square shape, which can be transformed into the printability parameter at three levels ( $Pr < 1$ ,  $Pr = 1$  and  $Pr > 1$ ) (Ouyang *et al.* 2016)

$$\text{Circularity, } C = \frac{4\pi A}{L^2} \quad \dots(2)$$

$$Pr = \left(\frac{\pi}{4}\right) * \left(\frac{1}{C}\right) = \frac{L^2}{16A} \quad \dots(3)$$

### Mechanical Properties of a 3D Printed Object

Texture analyser helps to measure hardness test of solid food objects and the Texture Profile Analysis (TPA) of semihard materials. The mechanical properties of 3D printed food structures, made of gels or purees or such soft materials can be evaluated using TPA. The important attributes to be generally calculated for gel like systems are adhesiveness, chewiness, springiness, hardness and gumminess (Rosenthal 2010). The mechanical properties of gellan gum, guar gum, xanthan gum, hydroxypropyl methylcellulose, locus bean gum, methylcellulose and gelatin were analysed by Kim *et al.* (2017) and found that the hardness of samples significantly increases by increasing the concentration. The impact of starch addition in the 3D construct made of lemon juice gels were observed by Yang *et al.* (2018a, b). The higher starch content results in notable increase of gumminess and hardness as a result of heavily compacted network bore by starch molecules.

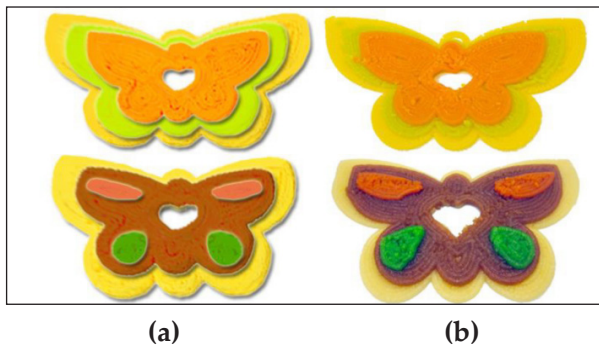
TPA fails when it comes to hard materials. In those cases, texture analyser can be used for measuring hardness of freeze dried or oven dried protein rich 3D printed structures (Lille *et al.* 2018). The snap



quality of printed chocolate objects has been studied by Mantihal *et al.* (2020) and found the importance of supports on snap force, with the help of texture analyser.

## MULTI-MATERIAL AND MULTI-PRINthead

There is a need of multi-printheads in the food printing sector for maintaining homogeneity as majority of printers have only one print head for extruding mixture of materials, which makes it difficult to control the distribution of materials within a layer. In this, platform controller activates each print head and thus gives information to individual layers and also controls its feed rate. Researchers had adopted multiple print heads Fab@home™ printers and created products including chocolate, muffins, cookie doughs, processed cheese etc. When it comes to multi-material printing, it is applicable only for a limited number of materials. Gray, (2010) solved this limitation by proposing the concept of electrospinning for producing multiple sub-components of food at a large scale.



**Fig. 6:** Multi-material food design and fabrication system (a) Multi-material food designs (b) Actual printed cookies

In spite of using multi printheads, it is really difficult to make a single platform for printing food types. Another approach to this is to create a macro material matrix with the help of mixing technique. This matrix enables to sustain homogeneity of material supply within each layer. This mixing technique can be agitated or static and can enable different material composition and making several material supply combinations (Nachal *et al.* 2019). Static mixing focusses on the force between the feed and force of friction between mixer's structure and the material. Agitated mixing provides better scope for changing the appearance and composition of

fabricated products (Sun *et al.* 2015c). Using multi-materials and multi-printheads are a new solution to mould food materials under multi scale into fascinating edible structures.

## ADVANTAGES AND LIMITATIONS OF FOOD PRODUCT FABRICATION WITH 3D PRINTING

Three-dimensional food printing has widely noticed by food enthusiasts all over the world as it has made so many hitherto impossible things possible. This technology made production of food seamless and fast. The major advantage is that it made possible to design the food so as to meet the concerns of individual related to their requirements and health conditions effectively and also it digitally manage the quality and quantity of food. In addition to this, it helps to reduce the waste output and improves sustainability of environment. This technology becomes eco-friendly and healthy since it converts various ingredients such as leaves from beets or insects, algae-based proteins into tasty food items. It also can utilize animal proteins which are developed in the laboratory or vegetable proteins to make meat substitutes that has high nutritional value and health benefits for intake.

In spite of these advantages, this technology faces certain constraints in fabricating 3D foods such as high cost of the printing machine, material suitability for printing, less embodiment of food-safe printing components, and lack of printing firmware (food based) and software applications (Kewuyemi *et al.* 2021). The advantages, limitations and suggestions for future work are explained in the following sub-heads.

### Available firmware and slicing software

In order to communicate the mechanical path of a 3D model, various printers with a firmware (built-in) and slicing software are used and it helps in accurate layer by layer deposition of materials. The mostly used slicing software packages, such as Repetier-Host, Slic3r, Cura, Simplify3D and Rhinoceros 3D, in order to circulate the planned mechanical route instructions and conditions of processing to 3D food printers are programmed for thermoplastic polymer printing. The elastic nature of printing material can result in certain inconsistencies in printing during deposition or after

the printing process. A digital 3D model solid phase (30g/100g), processing a doubles percentage volume fraction from 36% to 69% in wheat alternated with succulent insect printed snack has been reported. It is important to optimize the available slicing software for printing processes or a lasting way of creating slicing application packages for fabricating 3D foods (Guo *et al.* 2019).

### 3D Extrusion Printer Selection and Cost Implication

The development of 3D printing applications is limited by the factors such as the prohibitive cost, inflexibility of component-parts and proprietary restrictions (Cohen *et al.* 2009). But, the onset of RepRap open-source 3D printing machines has substantially overcome these challenges. Most of these printers are either uses a cartesian or delta configuration, and the cost is in the range of 160-6650 US dollars (Carolo, 2020). Recently, there are dedicated edible desktop printers including Foodbot 3D printers (China), byFlow Focus 3D printer (Netherlands), Natural Machines Foodini (Spain) etc. (Lansard 2021).

### Design of extrusion mechanism

Generally, the food printing extrusion mechanisms works on the basis of syringe driven (stepper motor or air pressure) or screw (driver: stepper motor) systems. A syringe's fusion consisting of a piston, tightly connected with a hose, which supplies compressed air for driving the loaded feed in a barrel(plastic) is depicted in the study of Liu and Cift, (2020). This kind of setup becomes a successful manipulation approach for 3D food printing. Guo *et al.* (2019) found out that the syringe-based printing system possess simple fluid characteristics with low pressure at the outlet of nozzle and high shear rate but the screw-based systems exhibit complex fluid characteristics with backflows between screw flight and barrel walls and a high shear rate. The existing printing systems can be upgraded from current batch-printing cycles to make remote food grade holding tanks of stainless steel, regulated over a set of conditions such as relative humidity and temperature for suitability of different food systems (Lanaro *et al.* 2017).

### Marketing of 3D Printed Foods

This is an attractive technology of food preparation and personalization engineered in pre-defined layers. The adaptability of processing various raw materials and increasing need of food items, fabricated using this technology are the key driving forces to industrial growth to attain an expected global 3D food printing market size of 1015.4 million US dollars in 2027 (Emergen Research 2021). Food Ink, is emerged as the first pop-up restaurant to provide meals made using 3D printers. Barilla, is the first Italian company, which developed the prototype of 3D printer for pasta (Savastano *et al.* 2018). Its spinout has introduced an E-commerce service, through which customers can order customized printed pasta online. This platform is auspicious for promoting large market share for novel 3D printed foods. Traditional cuisine with prior familiarity plays a crucial role in the sustainability of biomodified printable substrates (Kewuyemi *et al.* 2021). Furthermore, the printed healthy snacks have been accepted on the basis of their sensory attributes (Krishnaraj *et al.* 2019).

### CONCLUSION AND FUTURE WORK

3D food printing has proved its ability on making personalised chocolates or fabricating simple homogenous snacks. Anyhow, these applications are still basic with limited internal structures or monotonous textures. It is important to develop a systematic way to examine the printing materials, platform design, printing technologies and their influences on food fabrication. Meanwhile, the food designing process should be structured to encourage user's creativity, the fabrication procedure should be quantified to achieve consistency in fabrication results, and a simulation model should be developed to combine design and fabrication with nutrition control. With the evolution of an interactive open web-based user interface, 3D food printers can become part of an ecology system, where networked machines can order new ingredients, prepare favourite food item on demand and even collaborate with doctors to develop/customize healthier diets.

This study reviews about different food printing technologies, the various available printing materials, advantages and limitations of food product fabrication with 3D printing. From this



technology review, it can be seen that food printing may exert a significant influence on various types of food processing, which allow designers/users to manipulate/customize forms and materials with enhanced and unprecedented capability. This adaptability, applied to domestic catering or cooking service, can improve efficiency to provide high quality, freshly prepared food items to consumers, deliver personalized nutrition and enable users to develop new flavors, textures and shapes to create entirely new eating experiences.

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# A Study on Novel Approach to Sustainable Indian Agriculture - Precision Farming

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## ABSTRACT

Precision farming which is also regarded as site-specific management is reported as emerging approach that recognizes site-specific differences within-field and adjusts management action accordingly. Precision farming utilize the advanced technologies for efficient crop production and to minimize environmental impact caused due to over-usage of inputs. It is necessary to reduce the use of inputs by providing prescribed quantity and to increase the crop yield. Precision farming involves various tools such as Global Positioning System (GPS), Geographic Information System (GIS), Remote Sensing etc. for recording the precise data and making decision according to the stored data. In India, it is high time to adopt precision farming which leads to sustainable agriculture due to depletion of natural resources. It is stated that precision farming has scope for its adoption in small scale farms after considering its advantages and economics. The main purpose of this review is to understand the concept of precision farming and gaining knowledge about its adoption, economics, merits and obstacles.

## HIGHLIGHTS

- ① Precision farming should reach all the farmers and its implementation helps to overcome the depletion of natural resources and protection of environment.

**Keywords:** Precision farming, Site-specific management, Environmental impact, Adoption, Economics

Million years ago, man started agriculture as a profession and used basic resources like soil, water and air to strengthen his land and increased the agricultural productivity. These natural resources were degraded due to enormous increase in population and rapid industrialization and urbanization (Shanwad *et al.* 2004). Thus, innovation in agriculture is necessary to meet the demand and also due to use of technology and impacts of green revolution, degradation in environment is evident which should be reversed through sustainable farming. The advancement of technology and irrigation system lead to increase in productivity but at the same time higher use of agriculture inputs are not beneficial in increasing productivity. The management of all resources is essential for sustainability. Its high time to utilize all modern tools by combining the Information

Technology and Agriculture Science because of need for maximizing the input use efficiency, poor availability of labors and to improve economic and environmentally sustainable production. Before agriculture mechanization farmers used to monitor and fix the problems in the field mechanically but in recent days farmers are unable to adjust variabilities regarding yield potential, topography, nutrient deficiencies etc. (Robert *et al.* 2019). This necessitates the role of precision farming which is application of technologies and principles to manage variability linked with all aspects of agricultural production

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for improving both production and environmental quality.

Precision farming has emerged as current research topic at the end of 20<sup>th</sup> century all over the world (Hermann 2001). It is an integrated crop management system that meets the crop need in small areas within field. Precision farming can provide technology for eco-friendly agriculture and is more useful for farmers having small land holding in developing countries by supply of minimum inputs and receiving profitable outcome (Hakkim *et al.* 2016). Precision farming can be achieved by use of appropriate technologies for management and decision making. Adoption of precision farming technologies varies according to geography and technology used. Precision farming is more adopted in developed countries and there is requirement for overall adoption in large scale. However, the developing information technologies along with digitalization of agriculture sector are making adoption possible by increasing the degree of precision, expanding the applications, lowering the cost of inputs and easing the data processing. The profitability and efficiency of technologies will lead to the adoption of precision farming, also there is need for involvement of both public and private sectors in extension process for transferring the technology. The objective of our study is to provide basic information about precision farming and help out the new generation to be aware of this technology.

### **Precision Farming**

Precision farming is generally defined as an information and technology-based farm management system to identify, analyze and manage variability within fields for optimum profitability, sustainability and protection of land resource. The most suitable definition for precision farming in the context of Indian farming scenario could be precise application of agricultural inputs based on soil, weather and crop requirement to maximize sustainable productivity, quality and profitability (Singh, A. K. undated).

One generic definition is the kind of agriculture that increases the number of decisions per unit area of land per unit time with associated net benefits. Precision farming is a comprehensive approach to farm management and has following goals and

outcomes: increased profitability and sustainability, improved product quality, effective and efficient pest management, energy, water and soil conservation and surface and ground water protection. In other words, it is digital agriculture involving very large farm level mapping, comprehensive database creation on required resources generated through space-based inputs and field observation and making a detailed plan of work for maximizing the yield and reducing cost of inputs using decision support system.

Precision farming based on global positioning system (GPS) is the management of spatial and temporal variability at a sub-field level to improve economic returns and reduce environment impact. It involves both art and science to utilize advanced technologies for increasing crop production and minimize environmental impact. Precision agriculture is often defined by technologies that enable it and referred to as GPS (Global Positioning System) agriculture or variable rate farming or site-specific management (Patil *et al.* 2013). Generally precision farming is more feasible in large scale production. Variabilities in precision farming includes yield potentials, topography, soil characteristics, nutrient demands, biotic and abiotic stresses, cultivar selections, tillage practices and irrigation scheduling. Precision farming mainly aims to manage and distribute inputs on a site-specific basis rather than applying same amounts of inputs through field. It starts by noting the variations occurring in crop/ soil properties within the field, mapping and later management actions are taken as consequence of continuous investigation of spatial variability within that field (Rains *et al.* 2009). This recognizes, locates, quantify and records which can be managed by use of specific amounts of inputs at specific locations with the actual crop needs for small areas within a field. By understanding and dealing with natural variability found within a field can increase efficiencies through precision farming and better decision can be made about crop production aspects.

It is good to have better farm management practices before adopting precision farming because farmers should use information effectively. Both devices and information tend to increase returns to farmers. Maximum economic return can be achieved through precision farming compared to traditional



agriculture because in precision farming the field is divided into various management zones based on factors like soil pH, yield rates, pest infestation etc. and these zones are managed by application of required inputs using precision farming tools whereas in traditional farming field is treated as a homogenous area with whole field management practices and inputs are supplied uniformly throughout the field (Singh, A. K. undated). Precision farming can be summarized as technology that allows a farmer to follow site-specific management which is of doing right thing, in the right place, in the right time and in right way (Pierce and Nowak 1999). Precision farming provides a way to automate site-specific management using information technology, thereby making site specific management practical in commercial agriculture. Precision farming gained wide scope in production of major food grain crops like rice, wheat and also in high profit-making horticultural crops.

### **Need for Precision Farming**

The food production system of our country is majorly profited by Green Revolution in 1960's by making India self-sufficient. This was achieved due to high input application, increase in fertilization, irrigation, pesticides, higher use of high yielding varieties, increasing cropping intensity and increase in agricultural mechanization. Green revolution has made our country self sufficient but potential productivity of Indian high yielding varieties are lowest compared to world's crop yield level (Patil and Bhalerao 2013). The green revolution is also correlated with adverse impact on ecology. India's natural resources are degrading day by day with high input applications and it is reducing the originality of land. Major concern in agricultural growth and development are decline in the total productivity, diminishing and degrading natural resources (Healthy land and quality water both are becoming limited), stagnating farm incomes, lack of eco-regional approach, declining and fragmented land holdings, limited employment opportunities in non-farm sectors and global climate variation.

The challenges faced by global food system will likely to increase in future. This can be minimized immediately with the modern technologies and knowledge with sufficient will and investment. But it necessitates more radical changes to food

system and better investment in research to provide new solutions to novel problems. Due to limiting availability of natural resources, the adoption of precision farming is necessary. Precision farming potentiality is conceptualized through reduced use of water, fertilizers, herbicides and pesticides besides farm equipment for ecological and environmental benefits. Therefore, the adoption of newly emerged technology is one of the keys in future to increase agriculture productivity. There is a need to convert green revolution to evergreen revolution which can be achieved from precision farming where site-specific problems can be recognized within fields and management actions are managed accordingly.

### **Basic Steps in Precision Farming**

The variability in the field can be determined using the technologies, managing them with the use of agronomic practices and finally evaluating the outcomes. The three basic steps are explained in detail (Shanwad *et al.* 2004).

#### **1. Assessing variation**

The first and critical step in precision farming is assessing variation. To manage the variability, one should know about assessing it. Without proper assessment it is difficult to manage the variations. There is variation in elements and operations affecting the crop yield in accordance with space and time. Quantifying and determining variability of elements and operations by knowing the effect of different combinations for spatial and temporal variation is a huge challenge in precision farming. Techniques for assessing both spatial and temporal variation are available but they can proceed simultaneously. Temporal variation is different in different time which need observations at crop growth and development over growing season and this also includes spatial variability. Some variables are produced in space than time, making them more convenient to modern forms of precision management.

#### **2. Managing variability**

Management of variability is possible if the variability is assessed critically. Managing variability also includes both spatial and temporal variability. For example, the management of potassium and phosphorus is convenient because the temporal



variation of these nutrients is low. Likewise, management of nitrogen is difficult because the temporal variation is high. According to the assessed condition within field, farmers can implement agronomic practices which should be site-specific and accurate. Site-specific management can be effectively done with the use of various technologies making it easy and economical.

### 3. Evaluation:

Immediately after management it is important to evaluate the condition of field and outcomes. There are three important issues regarding evaluation of precision farming:

1. Economics
2. Environment
3. Technology

The analysis of profitability of precision agriculture involves the value that comes from application of data than from the use of the technology. Low usage of agrochemical, higher nutrient use efficiency and increased input efficiency are regarded as benefit to environment. As precision farming involves the application of technologies and agronomic principles for spatial and temporal variability management, transfer of technology to farmers is essential. The technology transfer differs for individual farms because of change in the problems related to each farm.

## Tools in Precision Farming

### 1. Global Positioning System (GPS) receivers

A GPS is a network of satellites and receiving devices that helps the users to record positional information while in motion at any time. This helps farmers by providing the exact position of field information such as soil type, pest occurrences, weed invasion, waterholes, boundaries, obstructions and crop measurements to be mapped (Singh, A. K. undated). This will locate specific spots in field which helps farmers to apply inputs in specific quantity to an individual field based on performance criteria and previous input applications. This system consists of light or sound guiding panel, antenna and receiver. While purchasing GPS, differential correction should be checked so that the signals could match the satellite signals in particular field.

### 2. Yield monitoring and mapping

Yield monitors are used to record yield data and create yield maps when linked with GPS. Yield monitors include several components like different sensors, data storage device, user interface and task computer (controls integration and interaction of above components). Yield monitors are important because yield data will enable to decide the management practices to be considered and to decide the effect of managed inputs such as fertilizers, seeds, agrochemicals and cultural practices (tillage and irrigation). Yield data of many years should be collected and considered for evaluation because yield data of single year cannot give adequate information as it may get affected by weather parameters along with field factors.

### 3. Geographic Information System (GIS)

GIS is hardware and software of computers and comprises of procedures that are designed to support the collection, storage, recovery and analysis of attributes and data of location to produce respective maps (Hakkim *et al.* 2016). It gained importance in agriculture for storing layers of information which include yield data, soil survey maps, rainfall, crops, soil nutrient levels and pests (Singh, A. K. undated). The recorded data can be displayed through visual perspective for interpretation. Other than storage and display of data, GIS can be used to evaluate present and alternative management by combining and manipulating data layers to produce an analysis of management scenarios. After analyzing the information can be used to compare and understand the relationships between the various factors affecting a crop on specific site.

### 4. Grid soil sampling and variable rate fertilizer (VRT) application

Grid soil sampling increases the intensity of soil sampling and uses the same principles of soil sampling. Soil samples are collected in a systematic grid includes location information that allows the data to be mapped. An application map of nutrient requirement is created through grid soil sampling. For each soil samples the crop nutrient needs are interpreted after analyzing the grid soil samples in laboratory. Variables rate technologies are automatic and applied to numerous farming

operations. Control computer, locator and actuator are three components of variable rate applicator. The fertilizer application map is plotted using the entire set of soil samples. According to the application map that is loaded into a computer mounted on a variable rate fertilizer spreader and a GPS receiver, the amount and kind of fertilizer product is applied to specific-site through product-delivery controller. This enables the land to get specific amounts of fertilizers at proper time (Hakkim *et al.* 2016).

## 5. Sensor technologies and remote sensing

Sensor technologies such as electromagnetic, conductivity, photoelectricity and ultrasound are one of the important tools of precision farming used to measure soil properties and plant fertility/ water status including humidity, vegetation, temperature, texture, structure, physical character, nutrient level, vapor, air etc. Remote sensing technology is useful tool for collecting numerous information simultaneously from a distance without laboratory analysis (Hakkim *et al.* 2016). These data are used to differentiate crop species, locate biotic and abiotic stress conditions, identify weeds, monitor drought, soil and plant conditions. Near-Infrared images are recorded in electronic cameras that are highly associated with healthy plant tissue. Location, extent and component of crop stress can be determined with the help of remotely-sensed images. A spot treatment plan that optimizes the use of agricultural chemicals is developed and implemented by using these images. Eventhough more information is gathered by remote sensing technology it is difficult to find key management factor because of varying field conditions such as timing and amount of nitrogen fertilizer application, timing and period of mid-season drainage and timing of harvest.

## 6. Precision irrigation in pressurized systems

GPS based controllers are used for controlling the irrigation machines for commercial use in sprinkler irrigation. Wireless communication and sensor technologies are being developed to monitor soil conditions to achieve higher water application efficiency and utilization by crop (Hakkim *et al.* 2016).

## 7. Identifying a precision agriculture service provider

The farmers are advised to consider the availability of custom services when making decisions about adopting site-specific crop management. Agricultural service providers or trained extension workers can offer a variety of precision farming services to farmers (Singh, A. K. undated). Intensive soil sampling, mapping and variable rate applications of fertilizer and lime are most common custom services. These custom services can decrease the cost and increase the efficiency of precision farming by providing capital costs for specialized equipment over more land and by using the skills of precision agriculture activities (Hakkim *et al.* 2016).

## 8. Rate controller

The devices designed to control the delivery rate of chemical inputs such as fertilizers and pesticides are rate controllers. These rate controllers monitor the speed of the tractor/sprayer traveling across the field, as well as the flow rate and pressure (if liquid) of the material, making delivery adjustments in real-time to apply a target rate. Rate controllers have been available for some time and are frequently used as stand-alone systems.

## Elements of Precision Farming

Mainly there are three elements in precision farming (Robert *et al.* 2009):

### 1. Information

Basic information or data is foremost needed for further proceedings in precision farming. In modern farming, collection of accurate data at proper timing by farmers is necessary for successful management. This information consists of crop characteristics, response of hybrids, soil properties, fertilizer requirements, weather predictions, weed and pest populations, plant growth responses, harvest yield, post-harvest processing and marketing projections. Farmers must be capable of finding, analysing, and using the available information at each step in the crop system. With the advancement and easy availability of data in recent years makes it possible to get within the requirement period. Data can be extracted from internet which is more accessible and quickly updated.



## 2. Technology

Farmers should know about the adaptation of new technologies for their operations. Personal computers can be used to store the data of past years and is accessed whenever required. These personal computers will organize, analyse and manage the data. Computer software including spreadsheets, databases, geographic information system (GIS) and other application software are readily available and easy to use. Global positioning system (GPS) can be used by farmers and agricultural consultants for locating position of field. Geographic information system (GIS) creates a field map by using the data recorded by Global positioning system (GPS) to assess the impact of farm management decisions.

Sensor technology is used to monitor soil properties, crop stress, growth conditions, yields or post-harvest processing are either available or under development and this instant information can be used to control operational inputs. Precision farming uses three general technologies: Crop, soil and positioning sensors – these include both remote and vehicle-mounted, “on-the-go” sensors that detect soil texture, soil moisture levels, crop stress, disease and weed infestations while the tractor travels along the field. Machine controls – these are used to guide field equipment and can vary the rate, mix and locate water, seeds, nutrients, or chemical applications. Computer-based systems – these include GIS maps and databases that use sensor information to prescribe specific machine controls.

## 3. Decision support or management

For best management prescriptions of crop production system, combination of traditional management practices and precision farming tools helps farmers. Most of the times it is difficult to understand the decision support system. Building databases based on the relationships between input and potential yields, refining analytical tools and increasing agronomic knowledge at the local level are yet to be accomplished. Agricultural researchers opined that decision support system is the least developed area in precision farming. Diagnostic and database development will eventually replace technologies as the real benefit of precision farming (Robert *et al.* 2009).

## Adoption in Small Scale Agriculture

The leading issue for agricultural scientist is feasibility of precision farming in small farms. The main point to be noted in precision farming is understanding the variability in the field and characterization of precision farming by variable management. At least two types of variability are observed, one is within-field variability and the other is between-field variability or regional variability (Shibusawa 2000). Each field is considered as unit on a map in case of between-field variability whereas with in- field variability focuses on a single field and cultivated with one plant variety. Considering the kind of variability is essential for precision farming in small farms. Precision farming should mean improved farm management no matter the farms are large or small. Ultimately, it should give higher economic return with a reduced environmental impact. Some low cost and low technology tools may be proved to be useful for small scale farms of developing countries (Mandol and Basu 2009).

Possible variable rate applications on a single small farm depends on understanding capacity, knowledge and skills of farmer. As the land size increases, precision farming should coordinate with different types of land use and many farmers having diverse motivations. Precision farming offers the possibility of developing a new kind of industry in view of development in rural area that includes small farms and local companies by fusing agriculture to various kinds of industrial activity. Hence, it is understandable that the size of farm in adoption of precision farming does not matter provided it depends on management strategy.

## Economic Studies of Precision Farming

The adoption of precision farming involves intensive capital investment and leads to greater expenditures on machinery and equipment as well as for access to information. Precision farming increases the quality of marketed goods. Product quality can be in two forms such as intrinsic and extrinsic quality. Intrinsic quality comprises of color, appearance, protein content, pesticide residue content, sugar content, starch content etc. Extrinsic quality is associated with production practices, origin or related aspects of the production process. Economic results of adoption of variable rate application methods





depends on the type of crop, field size and type of agriculture. Precision farming profitability is important for implementation of these technologies.

The benefits from precision farming are related to optimization of inputs, improvement of management and quality of work, crop yield improvements, minimizing cost through improved process control, reducing content of agrochemicals to the environment, environmental quality and business risk (Robert *et al.* 2019). None of the technologies adopted does not provide a total solution until and unless it is used extensively for commercial purpose. The coordination between private and public sectors can lead to the economic growth of precision farming by developing and implementing technologies, market development responsibility and customer satisfaction. Economic studies related to profitability are nuclear and non-comparable.

### Merits of Precision Farming

- ♦ *Agronomical perspective:* Use agronomical practices by looking at specific requirement of crop.
- ♦ *Technical perspective:* Allows efficient time and management.
- ♦ *Economical perspective:* Increases crop yield, quality and reduces cost of production by efficient use of farm inputs, labor, water etc.
- ♦ *Environmental perspective:* Ecofriendly practices in crop.
- ♦ *Managing spatial variability for decision making:* Precision farming provide complete field related information through direct sampling.
- ♦ *Management of environmentally sensitive areas:* By calculating the greenhouse gas emissions due to mechanized operations, they can limit emissions to the environment.
- ♦ *Precise nutrient applications:* Yield maps can be used to alter fertilizer applications that suits current soil characteristics.
- ♦ *Precise pesticide application:* Specific amounts of pesticides are applied to specific site and it also prevent overlapping during spraying by the use of light bar guidance system. This will reduce the use of inputs and decrease adverse effect on environment.

- ♦ *Automations using Guidance Systems:* Direct economic benefits are achieved using automated unmanned systems (reduced labour costs) and reduced impact on the environment (reduction of machinery pass frequency and reduction of soil compaction) (Thomas and Georgios 2017).

### Obstacles in Adopting Precision Farming

Common obstacles for adoption of precision farming in developing countries like India are as follows (Shanwad *et al.* 2004):

- ♦ Culture and perceptions of the users.
- ♦ Small farm size.
- ♦ Lack of success stories.
- ♦ Heterogeneity of cropping systems and market imperfections.
- ♦ Landownership, infrastructure and institutional constraints.
- ♦ Lack of local technical expertise.
- ♦ Knowledge and technical gaps among farmers.
- ♦ Data availability, quality and costs.

### CONCLUSION

Precision Farming could be a solution to threats associated to low agricultural productivity and environmental impact. In developing countries, it has not reached every farmer due to lack of transfer of technology and unclear economic framework. Indian farmers with the help of public and private sectors will be able to adopt this technology as well as can deal with the severe problems faced by them such as low outputs, depletion in water levels, increasing cost of inputs only if they are trained about technologies and providing detailed information about precision farming. Precision farming can be adopted in various fields such as agriculture, horticulture and livestock production which will help farmers to increase their economic returns and making India self-sufficient in food production.

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# Study of Heterosis, Residual Heterosis and Inbreeding Depression in Two Crosses of Tomato

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## ABSTRACT

Heterosis, which is hybrid vigour that results in an increase in fruit yield with early development and higher quality, is one of the most essential approaches to improve yield and quality attributes. Heterosis is a natural phenomena in which the physical and functional traits of hybrid offspring of genetically heterogeneous individuals improve over time. The goal of this research is to find out how much hybrid vigour and inbreeding depressions, as well as residual heterosis, are present in two tomato crosses (DMT-2 LINE-38 and LINE-33-1 LA-1). A population of two crosses was assessed with 14 parameter test of generation mean analysis. The significant and negative heterosis (heterobeltiosis) was depicted for days to first harvest in cross I with negative inbreeding depression, which indicates desirable earliness found in those families. The heterosis, as well as inbreeding depression was observed in the desired direction in cross I and II for fruit yield per plant and for quality *viz.*, pericarp thickness, pH of fruit juice and TSS indicating possibilities to get the desired segregants in a further breeding program.

## HIGHLIGHTS

- Tomato (*Solanum lycopersicum* L.) is one of the most important solanaceous vegetable crops all over the world. It was introduced in India by English traders of East India company in 1822 (Kalloo 1988). The tomato originated in a wild form in Ecuador, Peru, and Bolivia of South America (also known as the center of diversity of wild tomato). Globally, it is grown in an area of 5.02 million hectares with the production of 170.75 million tonnes and productivity of 33.99 tonnes per hectare (FAO, 2020). China, India and USA are the major tomato producing countries. In India, it is grown in an area of about 0.88 million hectares with production of 18.74 million tonnes and the average productivity is about 21.24 tonnes per hectare. The important tomato growing states are Andhra Pradesh, Karnataka and Madhya Pradesh. In Karnataka, tomato is grown on an area about 0.06 million hectares with production of about 2.13 million tonnes with the productivity of about 31.54 tonnes per hectare (Anon., 2019).

**Keywords:** Generation mean analysis, Heterosis, Heterobeltiosis, Residual heterosis, Inbreeding depression

Tomato is consumed year-round and its importance is mainly derived in two forms: used as a fresh vegetable as well as an important source for the processing industry. At present time, superior quality and adequate quantity of vegetables for commercial agro-processing are not being grown sufficiently. Cultivation of tomatoes began to decline during the last few years, which requires conventional and scientific efforts to increase the production per unit area to compensate for

the shortfall in the cultivated area. Many local farmers grow average yielding varieties, which are characteristically low yielding and of poor quality for the traits such as high-water content, poor color and low Brix content against the increasing

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demand at the local and international levels for superior quality. To overcome these problems, the development of high yielding and superior quality varieties of tomato is imperative in the cultivation to meet the total market demand. Since the 1980s, the emphasis of new cultivar development has been focused on the production of  $F_1$  hybrids (Grandillo *et al.* 1999). Generally, hybrids are preferred over pure line varieties in tomatoes due to their superiority in terms of yield as well as the quality of fruit. Hybrid breeding technology is greatly applied in cross-pollinated crops and is limited in autogamous crops due to the strict genetic makeup of the plant and floral biology. Commercial exploitation of heterosis in self-pollinated crops has been limited owing to technical difficulties involved in hybrid seed production. Tomato is a self-pollinated crop with hermaphrodite flower and can be easily emasculated for crossing technique, therefore it has a suitable mechanism to produce hybrid seed at a commercial scale. However, it adds a higher labour cost to the total production cost.

Heterosis increases yield and quality in many crops and vegetables and it has been intensively used in plant breeding. The identification of superior parental combinations that provide high heterosis for yield and quality is the most important factor in hybrid development. Heterobeltiosis is useful in the identification of promising cross combinations for the improvement of the crop through conventional breeding strategies. It may lead to an increase in yield, reproductive ability, adaptability to general vigour, different biotic and abiotic stresses and also improve fruit quality. Contrarily, inbreeding depression leads to decreased fitness and vigour due to the expression of lethal and sublethal alleles which are generally masked under heterozygosity. It leads to increased homozygosity and fixation of undesirable recessive genes in  $F_2$  and successive generations, while in the case of heterosis, favorable dominant genes of one parent are masking the effect of harmful recessive genes of another parent.

Molecular, genetic, and physiological mechanisms underlying this phenomenon are not well understood yet (Birchler *et al.* 2010). The demand for tomatoes is increasing day by day but their production and quality is affected by many diseases, stresses and many other factors. A considerable amount of important work has already been done in this crop,

but a great amount of information is still needed for the understanding of the genetics of fruit yield, yield attributing traits, and quality parameters of this crop. The purpose of this study was to estimate the heterosis of yield-related traits as well as inbreeding depression and residual heterosis in the  $F_2$  generation. The experimental material is constructed in such a way that the estimation of the heterosis, inbreeding depression, and residual heterosis effect in a respective generation is possible.

## MATERIALS AND METHODS

The experimental material comprises of two crosses, each representing six generations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $B_1$ , and  $B_2$ ) were raised in a compact family block design in field trials. Two crosses were derived from four parents *viz.*, DMT-2, LINE-38, LINE-33-1 and LA-1 with hand emasculation and pollination. Observations for the different traits under study were recorded on randomly selected and tagged plants from each experimental unit and each replication *i.e.*, five plants from each  $P_1$ ,  $P_2$ , and  $F_1$  and 300 hundred plants from each  $F_2$  generation and 120 plant for back cross generation was recorded. The mean of  $F_1$  hybrids and  $F_2$  generation over replication were utilized for the estimation of heterosis, inbreeding depression, and residual heterosis.

**1. Estimation of Heterosis.** Heterosis expressed as a percent increase or decrease of  $F_1$  hybrid over its better parent value (BP) and mid parent heterosis (MH) was computed using the following formulae.

**1. Heterosis over better parent (Heterobeltiosis) HB (%)**. The heterosis over better parent was calculated as per the Fonseca and Patterson, (1968).

$$HB(\%) = \frac{\bar{F}_1 - \overline{BP}}{\overline{BP}} \times 100$$

Where,  $F_1$  = Mean value of  $F_1$  hybrid *i.e.*  $F_1$

$BP$  = Mean performance of better parent.

**2. Mid parent heterosis MH (%)**. The heterosis over average of two parents was calculated as per the Meredith and Bridge, (1972).

$$MP(\%) = \frac{\bar{F}_1 - \overline{MP}}{\overline{MP}} \times 100$$

Where,  $MP$  = Mean performance of two parents

### 3. Estimation of inbreeding depression (ID %).

Inbreeding depression was computed by using the following formula,

$$\text{Inbreeding depression (\%)} = \frac{\bar{F}_2 - \bar{F}_1}{F_2} \times 100$$

**4. Estimation of Residual Heterosis (%).** The residual heterosis from  $F_2$  generation was worked out as per the formula given below:

$$\text{Residual heterosis} = \frac{\bar{F}_2 - \overline{BP}}{\overline{BP}} \times 100$$

## RESULTS AND DISCUSSION

The results observed for heterosis over mid parent and better parents, residual heterosis as well as inbreeding depression for 14 traits are summarized in Table 1.

The measurement of heterosis over mid parent is of academic importance for studying genetics of heterosis but has limited practical usefulness. On the other hand, the heterosis measured over better parent is of much practical importance. The commercial exploitation of heterosis is considered to be an outstanding application of principles of genetics into the field of plant breeding. Heterosis has been successfully exploited in many crops like tomato, bajra, maize, bitter melon, sorghum, castor, cotton, bottle gourd and many other vegetable crops. In present study, heterosis over mid as well as better parents, Residual heterosis and inbreeding depression was estimated (Table 1).

The heterosis over better parent (heterobeltiosis) for different traits was ranging from -2.19 to 9.26 % and highest heterobeltiosis was recorded in average fruit weight (9.26 % in Line-33-1 × LA-1), mean-while heterosis over mid parent ranges from -1.21 to 13.26 %. The highest heterosis over mid parent recorded in average fruit weight (13.26 % in Line-33-1 × LA-1).

For the character days to 1<sup>st</sup> harvest the low scoring parent was taken as better parent. The degree of heterosis over better parent as well as mid parent varied from cross to cross for all the 14 characters.

Significant and positive heterosis over better parent observed for plant height (9.22 % in DMT-2 × Line-38 and 7.22 % in Line-33-1 × LA-1), number of fruits per plant (7.5 % in DMT-2 × Line-38), polar length of

fruit (4.99 % in Line-33-1 × LA-1), pericarp thickness (0.50 % in Line-33-1 × LA-1), number of locules (0.55 % in DMT-2 × Line-38), total soluble solids (0.80 % for DMT-2 × Line-38 and 0.65 % in Line-33-1 × LA-1), pH of fruit juice (0.30 % in DMT-2 × Line-38).

Similarly significant and positive heterosis over mid parent was observed for plant height (6.37 % in Line-33-1 × LA-1), primary branches per plant (0.25 % in DMT-2 × Line-38), number of clusters per plant (2.47 % in DMT-2 × Line-38), number of fruits per cluster (0.27 % in DMT-2 × Line-38 and 0.42 % in Line-33-1 × LA-1), number of fruits per plant (9.72 % in DMT-2 × Line-38), average fruit weight (13.26 % in Line-33-1 × LA-1), polar length of fruit (5.61 % in Line-33-1 × LA-1 and 2.91 % in DMT-2 × Line-38), equatorial length of fruit (4.59 % in DMT-2 × Line-38 and 3.56 % in Line-33-1 × LA-1), pericarp thickness (0.69 % in Line-33-1 × LA-1 and 0.64 % in DMT-2 × Line-38), number of locules per fruit (0.78 % in DMT-2 × Line-38 and 0.42 % in Line-33-1 × LA-1), total soluble solids (1.02 % in DMT-2 × Line-38 and 0.85 % in Line-33-1 × LA-1), pH of fruit juice (0.41 % Line-33-1 × LA-1 and 0.4 % in DMT-2 × Line-38) and yield per plant (0.50 % in DMT-2 × Line-38 and 0.47 % in Line-33-1 × LA-1). The positive and significant heterosis over mid parent as well as better parent found undesirable for days 1<sup>st</sup> harvest in the above mentioned crosses but their magnitude were very less could be neglected. Significant and negative heterosis over better parent observed in days to 1<sup>st</sup> harvest (-2.19 % in DMT-2 × Line-38), primary branches per plant (0.3 % in Line-33-1 × LA-1). Significant negative mid parent heterosis was not observed for any characters.

Heterosis in desirable direction for various traits in tomato was reported by several research workers such as Shekar *et al.* (2010), Shankar *et al.* (2013), Kumar *et al.* (2018), Madhavi *et al.* (2018) and Barragan *et al.* (2019), for total fruit yield per plant; Joshi *et al.* (2015), Zengin *et al.* (2015) and Kumar *et al.* (2018) for plant height, fruit yield, number of fruits plant, fruit length and average fruit weight and for earliness (days to 1<sup>st</sup> harvest); Kumar *et al.* (2018).

Residual heterosis is the amount of heterosis shown by  $F_2$  and subsequent segregating generations. Residual heterobeltiosis and economic heterosis in  $F_2$  generations was estimated as the percentage of deviation of generation mean of  $F_2$  from better parent

**Table 1:** Estimates of heterosis over mid parent (MP), heterobeltiosis (BP), residual heterosis (RH) and inbreeding depression (ID) for 14 characters in two crosses of tomato

	Crosses	Days to 1 <sup>st</sup> harvest	Plant height (cm)	Primary branches per plant	Number of clusters	Number of fruits per clusters	Number of fruits per plant	Average fruit weight (g)
Mid parent heterosis	DMT-2 × Line-38 Line 33-1 × LA-1	-1.21 ± 0.67 0.54 ± 0.1	2.71 ± 2.41 6.37* ± 2.28	0.25* ± 0.11 -0.1 ± 0.06	2.47* ± 1 1.8 ± 1.02	0.27** ± 0.1 0.42* ± 0.13	9.72* ± 2.45 2.42 ± 2.7	5.31 ± 2.82 13.26* ± 5.18
Better Parent heterosis	DMT-2 × Line-38 Line 33-1 × LA-1	-2.19* ± 0.69 0.1 ± 0.69	9.22** ± 2.39 7.72* ± 2.46	0.15 ± -0.12 -0.3* ± 0.09	0.92 ± 1.18 0.37 ± 1.12	0.22 ± 0.12 0.15 ± 0.15	7.5* ± -2.97 2.88 ± 3.18	3.1 ± 3.23 9.26 ± 5.49
Residual Heterosis	DMT-2 × Line-38 Line 33-1 × LA-1	-41.02** 892.47**	7.29** 8.84**	6.87** -4.82**	11.64** 36.32**	10.36** 18.04**	2.95 9.98**	19.19** 5.66
Inbreeding depression	DMT-2 × Line-38 Line 33-1 × LA-1	-1.49 ± 0.62 -1.47** ± 0.50	4.62* ± 2.24 5.20* ± 2.21	0.29* ± 0.1 -0.02 ± 0.05	4.02* ± 0.92 1.46 ± 0.87	0.04 ± 0.09 0.05 ± 0.12	8.14** ± 5.06 2.27 ± 2.41	1.45 ± 2.92 2.5 ± 5.3
	Crosses	Polar length of fruit (mm)	Equatorial length of fruit (mm)	Pericarp Thickness (mm)	Number of locules	TSS (° brix)	pH of fruit juice	Yield per plant (kg)
Mid parent heterosis	DMT-2 × Line-38 Line 33-1 × LA-1	2.91** ± 1.03 5.61** ± 0.8	4.59** ± 1.49 3.56** ± 1.04	0.64** ± 0.08 0.69** ± 0.11	0.78** ± 0.21 0.42* ± 0.2	1.02** ± 0.07 0.85** ± 0.10	0.4** ± 0.08 0.41** ± 0.09	0.50** ± 0.13 0.47** ± 0.12
Better Parent heterosis	DMT-2 × Line-38 Line 33-1 × LA-1	1.37 ± 1.16 4.99** ± 0.88	3.04 ± 1.62 1.91 ± 1.13	0.23* ± 0.07 0.50** ± 0.12	0.55* ± 0.22 0.12 ± 0.25	0.80** ± 0.08 0.65** ± 0.13	0.30** ± 0.10 0.13 ± 0.09	0.31 ± 0.16 0.24 ± 0.15
Residual Heterosis	DMT-2 × Line-38 Line 33-1 × LA-1	29.01** 7.04**	13.82** 21.07**	16.8** 6.41**	7.15** 38.64**	4.37** 6.16**	13.40** 33.78**	4.23** 6.24**
Inbreeding depression	DMT-2 × Line-38 Line 33-1 × LA-1	3.54** ± 0.9 1.99* ± 0.73	4.42** ± 1.43 1 ± 0.94	0.34** ± 0.06 0.52** ± 0.09	1.06** ± 0.19 0.14 ± 0.17	0.89** ± 0.06 0.64** ± 0.09	0.3* ± 0.11 0.09 ± 0.08	0.43* ± 0.11 0.36** ± 0.11

\*, \*\* Significant at 5 and 1 % levels, respectively



and standard check value respectively Residual heterosis was found positive and significant for most of the traits except number of fruits per plant of cross I and average fruit weight of cross II with the range of -41.02-892.47. Similar findings were observed by Damor *et al.* (2021) and Kumar and Singh (2016).

In the present study, either low or moderate amount of inbreeding depression (ID) in desirable direction was found in most of the traits. The inbreeding depression in two crosses was within the range of -1.49 % to 8.14 %. The character which manifested low heterosis in  $F_1$  also showed low inbreeding depression in  $F_2$ . The significant and negative heterosis found in days to 1<sup>st</sup> harvest (-1.49 % in DMT-2 × Line-38 and -1.47 % in Line-33-1 × LA-1) indicating that  $F_1$ 's matured earlier than their respective  $F_2$ 's.

It is desirable to have highly significant and positive heterosis over better parent with low inbreeding depression for traits like fruit yield and its component characters such as plant height, number of clusters per plant (Line-33-1 × LA-1), number of fruits per cluster (DMT-2 × Line-38 and Line-33-1 × LA-1), number of fruits per plant (Line-33-1 × LA-1), average fruit weight (DMT-2 × Line-38 and Line-33-1 × LA-1), equatorial length of fruit (Line-33-1 × LA-1), number of locules (Line-33-1 × LA-1). The magnitude of inbreeding depression in the present investigation varied from cross to cross indicating influence of genetic constitution of cross. Similar results were found by Singh *et al.* (1996) and Kumar and Singh (2016) reported considerable inbreeding depression in the  $F_2$  generation for plant height, days to 1<sup>st</sup> harvest, fruit length and width and fruit yield per plant; Dagade *et al.* (2015) observed significant inbreeding depression for number of fruits per plant, fruit yield per plant and total soluble solids; Avidokos *et al.* (2021) found high degree of inbreeding depression for pericarp thickness and number of locules per fruit.

## CONCLUSION

Plant breeding has achieved tremendous success in the development of hybrid cultivars, particularly in self-pollinated crop plants. For fruit yield, earliness, and quality attributes, the examination of available tomato germplasm demonstrated a considerable heterotic influence. Days to first

harvesting, plant height, number of fruits per plant, number of fruits per cluster, average fruit weight, polar and equatorial length, number of locules, TSS, pH of fruit juice, and yield per plant showed the highest and significant heterosis in the desired direction; however, residual heterosis was observed for the majority of the characters. In most of the crosses, heterobeltiosis/heterosis over midparent was substantial for main traits, showing the importance of hybrids for commercial crop genetic gain exploitation. The presence of overdominant gene activity is represented by estimations of substantial inbreeding depression with significant heterosis. The  $F_2$  generation's estimates of low inbreeding depression show a decrease in mean. Low inbreeding depression, on the other hand, lets the breeder to produce pure lines by using long selfing cycles. More emphasis on hybrid breeding can be made to improve self-pollinated crops by adding more male sterility and apomictic genes.

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# A Critical Study on the Present Status and Scope of Natural Farming in the State of Andhra Pradesh, India

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## ABSTRACT

Natural Farming is a chemical-free alias traditional farming method. It is considered as agroecology based diversified farming system which integrates crops, trees and livestock with functional biodiversity. In India, Natural farming is promoted as Bharatiya Prakritik Krishi Paddhati Programme (BPKP) under centrally sponsored scheme- Paramparagat Krishi Vikas Yojana (PKVY). Government of Andhra Pradesh expanded zero budget natural farming in 2015. Andhra Pradesh's Department of Agriculture appointed Rythu Sadhikara Samstha (RySS) to oversee the Climate Resilient Zero Budget Natural Farming program. RySS, a state-run research institute, was established to train farmers and promote farmer-to-farmer learning. The state launched ZBNF as a pilot program with over 700 villages and approximately 40,650 farmers in 2016 (RySS 2019). As of March 2020, approximately 623,300 farmers were enrolled in Andhra Pradesh's ZBNF program and the total amount of land cultivated under ZBNF was almost three percent of total net sown area in the state (181,600 hectares). By 2027, Andhra Pradesh plans to expand ZBNF to all 6 million farmers and 8 million hectares. The ZBNF program is funded by the national and state government through Paramparagat Krishi Vikash Yojana and Rashtriya Krishi Vikash Yojana programs, as well as other partners, including the Azim Premji Philanthropic Initiative, the International Fund for Agricultural Development, and the Bill & Melinda Gates Foundation. The cost to RySS to transition one farmer to ZBNF is approximately 25,550 INR (348 USD), where 73 percent of the cost is dedicated to capacity building. Recent agricultural graduates are also stationed in a village for two years as Natural Farming Fellows where they lease land and practice ZBNF to demonstrate its viability to nearby farmers. Natural Farming Fellows adapt ZBNF's core elements to local conditions and help to attract youth to the program. Women's self-help groups are instrumental in scaling up ZBNF. As of 2019, more than 161,000 women's self-help groups were mobilizing farmers to adopt ZBNF, preparing farming plans, marketing ZBNF locally, and monitoring farmers' progress. RySS relies on women's self-help groups to identify landless farmers to participate in kitchen gardens etc.

## HIGHLIGHTS

- To know the present status of natural farming in the state and as well as different stakeholders engaged in the practice of natural farming promotion in the state. Benefits as well as constraints faced by the natural farming farmers and what are the condition to be set up to increase the area of natural farming in the state.

**Keywords:** Chemical free farming, Debt free farmers, Natural farming fellows, Women SHGs, BPKP-PKVY

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Government of Andhra Pradesh expanded zero budget natural farming in 2015. Andhra Pradesh's Department of Agriculture appointed Rythu Sadhikara Samstha (RySS) to oversee the "Climate Resilient Zero Budget Natural Farming" program. RySS, a state-run research institute, was established to train farmers and promote farmer-to-farmer learning. The state launched ZBNF as a pilot program with over 700 villages and approximately 40,650 farmers in 2016 (RySS 2019). As of March 2020, approximately 623,300 farmers were enrolled in Andhra Pradesh's ZBNF program (almost 10.5 percent of all farmers in Andhra Pradesh), and the total amount of land cultivated under ZBNF was almost three percent of total net sown area in the state (181,600 hectares) (Khurana and Kumar 2020). By 2027, Andhra Pradesh plans to expand ZBNF to all 6 million farmers and 8 million hectares (RySS 2019). The ZBNF program is funded by the national and state government through Paramparagat Krishi Vikash Yojana and Rashtriya Krishi Vikash Yojana programs, as well as other partners, including the Azim Premji Philanthropic Initiative, the International Fund for Agricultural Development, and the Bill & Melinda Gates Foundation (Mishra 2018; RySS 2019). The cost to RySS to transition one farmer to ZBNF is approximately 25,550 INR (348 USD), where 73 percent of the cost is dedicated to capacity building (RySS 2019). Farmers do not receive a subsidy from the government to adopt ZBNF (Gupta and Jain 2020). ZBNF's rapid expansion in the state can be attributed to the program's extensive network of fellows to recruit and train farmers, along with its strategic linkage with women's self-help groups. In each village where the program is active, 50 Community Resource Persons (CRPs), who have previously been identified as "champion farmers" for their success with ZBNF, train other farmers in their community, provide marketing support, and collect data for RySS (RySS 2019).

Recent agricultural graduates are also stationed in a village for two years as Natural Farming Fellows where they lease land and practice ZBNF to demonstrate its viability to nearby farmers. Natural Farming Fellows adapt ZBNF's core elements to local conditions and help to attract youth to the program (Gupta and Jain 2020). Women's self-help groups are instrumental in

scaling up ZBNF. Millions of women in self-help groups across Andhra Pradesh collectively invest their own savings, loans, and government grants into their communities (Deshmukh-Ranadive 2004). As of 2019, more than 161,000 women's self-help groups were mobilizing farmers to adopt ZBNF, preparing farming plans, marketing ZBNF locally, and monitoring farmers' progress (RySS 2019). RySS relies on women's self-help groups to identify landless farmers to participate in kitchen gardens or lease land (Gupta and Jain 2020). Women's self-help groups are also a source of financial capital for farmers during their transition to ZBNF (Gupta and Jain 2020). ZBNF is promoted as a solution to the farmer debt crisis and environmental degradation in Andhra Pradesh and throughout other states in India as well. Himachal Pradesh is promoting ZBNF under the state-sponsored scheme, "Prakritik Kheti Khushal Kisan" (Department of Agriculture Himachal Pradesh). ZBNF is also practiced in Karnataka, Kerala, Haryana, and Gujarat (Khurana and Kumar 2020), and continues to attract international attention as a burgeoning agroecological movement.

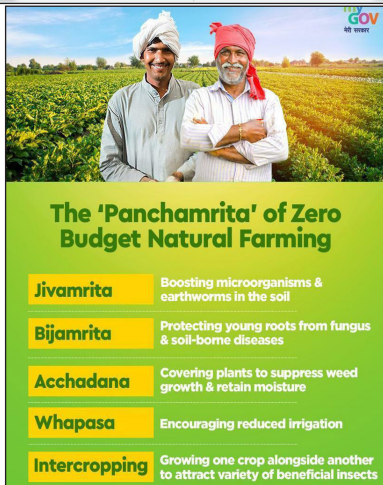
## Pillars of ZBNF

Concerning soil fertility, soil microbes play an important role, they involve in a nutrient cycle like C & N cycle which are required for plant growth (Lazarovits 1997).

### 1. Jivamrita/Jeevamrutha

Microorganisms play an important role in the conversion of unavailable forms of nutrients to available form in the plant root zone. The microbes present in jeevamrutha helps non-available form to dissolved form when it is inoculated into the soil. It also helps as antagonism to (biological control) pathogens (Glick & Bashan, 1997). PGPR, cyanobacteria, and Solubilizing Bacteria (PSB), mycorrhizal fungi, Nitrogen-fixing bacteria are some important microbes present in the product (Chen *et al.* 1995). It requires 20 kg cow dung, 5-10 l urine, 2 kg dicot flour are well mixed and this add-in irrigation tank at regular intervals of 15 days until the soil is enriched or spray 200l of jeevamruth twice in a month. It provides nutrients, microbial population, and helps to prevent fungal and bacterial plant diseases. It requires only 1st three

years cycle after that system that self-sustaining. According to Mr. Palekar, only one cow is needed for 30 acres of land that should be a local desi cow not imported Jersey or Holstein because of imported cow dung and urine contains more pathogens and desi cow dung contains 300 to 500 crores of effective beneficial microbes.



## 2. Bijamrita

It is composed of 20 l water, 5 kg cow dung, 5l urine 50 g lime, and a hand full of soil are thoroughly and store in a tank. It is used as a seed treatment, contains naturally occurring beneficial microorganisms. Research studies showed that inoculating with bijamrith to protect the crop from harmful soil-borne pathogens and young seedlings roots from fungus and soil-borne and seed-borne diseases also help to produce IAA and GA3 (Sreenivasa *et al.* 2010).

## 3. Acchadana/Mulching

Mulching is of three types followed, they are straw mulch, soil mulch, and live mulch. The growth of cover crops like legumes helps to reduce the weed population and increases water infiltration capacity.

By their root nodules fixes atmospheric N into the soil which helps N supply to crops. From these residues retention on the surface of soil increases the microbial degradation process and liberation of N from nitrification. It also supplies organic matter to the soil which contains many micro and macronutrients. Improves seed germination without soil plowing, reduce soil temperature in extreme condition, and increase soil temperature during winter. It conserves soil moisture by reducing evaporation loss of water from the soil layer and retains water for a longer time.

## 4. Whapasa –aeration

The main concern here is conserving water and the precise application of water-based on crop water requirement. Application of water in alternative furrows because of all roots of plants not absorb efficiently, younger horizontal and vertical roots absorb more amount of water than older one and nutrients by older roots. In soil, out of soil mineral and organic matter, there is an equal proportion of water and air present. If a higher amount of water application leads to hold air space in the soil and plant suffers oxygen deficiency it may lead to cause death of plants except water-loving plants like rice. The soil aeration also an important parameter to plant growth so application interval should be longer.

## Pest management in ZBNF

Crops are damaged by pests and diseases about most of the yield loss by weeds followed by pests and diseases. Controlling of this loss is also a big challenge in natural farming. Plant extractions are used to make a compound that kills or controls the pest population in the crop field. Some of plant protections are made by using a mixture of butter milk, cow milk, pepper powder, neem seed and green chilli (Palekar 2016). Some research papers found some naturally extracted chemical-free compounds are explained below. They are—

### 1. Agriastra

It consists of local cow urine (10l), tobacco leaves (1 kg), green chili (500 g), local garlic (500 g), and neem leaves pulp crushed in cow urine (5l) store it in a cool place. Take 2l per 100l of water and spray



on crops. It effectively controls the pests like Leaf Roller, Stem Borer, Fruit borer, Pod borer.

## 2. Brahmastra

It is 2<sup>nd</sup> way to control the pest population in natural farming, it can be made by collecting different plant leaves like neem leaves, custard apple leaves, lantern camellia leaves, guava leaves, pomegranate leaves, papaya leaves, and white datura leaves are crushed and boiled with urine finally make filtration. After filtration, the extractant can store for longer use. It is most effective against all of the sucking pests, pod borer, fruit borer, etc.

## 3. Neemastra

By using 5 l of local cow urine, 5 kg cow dung, 5 kg neem leaves 5 kg of neem pulp mixed well, and keep to airtight for 24 hours for fermentation. After the fermentation process is ready to use. Mainly controls sucking pests & Mealy Bug.

## ZBNF ADOPTION

**Partial adoption:** Some farmers reduce the risks associated with adopting ZBNF by experimenting with only a subset of ZBNF practices, most commonly jeevamrutham and beejamrutham (Gupta *et al.* 2020). This incremental adoption of ZBNF practices is referred to as a vertical transition (Gupta *et al.* 2020) and also as stacking (especially when there are two or more practices implemented at the same time). Some farmers also choose to adopt ZBNF on only a portion of their land. RySS expects that a farmer will typically adopt ZBNF on a quarter of their landholding in the first year, half of their landholding in the second year, and complete adoption during the third year—a process described as a horizontal transition (Gupta *et al.* 2020; RySS 2019). As a result, the land area under ZBNF adoption is lower than the total amount of land associated with farmers in the ZBNF program.

**Farmer characteristics:** Farm size and education are the most predictive farmer characteristics for ZBNF adoption. Most ZBNF farmers have small to medium sized landholdings (Gupta *et al.* 2020; Khadse *et al.* 2018). The Council on Energy, Environment, and Water (CEEW) surveyed 581 farmers (254 ZBNF, 327 non-ZBNF) in six districts in Andhra Pradesh. More than 70 percent of ZBNF farmers surveyed were

marginal farmers (owning or renting less than 2.5 acres), approximately 20 percent were small farmers (2.5 to 5 acres), and the remaining percentage were large farmers (more than 5 acres) (Gupta *et al.* 2020). In Karnataka, approximately 72 percent of ZBNF farmers surveyed owned less than 10 hectares (Khadse *et al.* 2018). On average, ZBNF farmers received more education than non-ZBNF farmers. Forty-two percent of non-ZBNF farmers surveyed in Gupta *et al.* (2020) did not receive any formal schooling, compared to only 20 percent of ZBNF farmers (Gupta *et al.* 2020). Based on a survey of 60 farmers in Andhra Pradesh, one study concluded that education has a positive, statistically significant impact on farmers' perception of ZBNF (Sarada and Kumar 2018). A small proportion of women relative to men adopt ZBNF, although more than 161,000 women's self-help groups form the foundation of the program (RySS 2019). Gupta *et al.* (2020) found that 4 and 7 percent of conventional and ZBNF farmers surveyed, respectively, were women. Women in Andhra Pradesh typically participated in the ZBNF program by selling inputs, marketing to other farmers in the community, and monitoring farming plans (RySS 2019; Tripathi *et al.* 2018). Studies did not observe significant differences between ZBNF and non-ZBNF farmers by caste. Scheduled Caste and Scheduled Tribe farmers represent the smallest share of ZBNF farmers, followed by the general or Other Backward Class (Gupta *et al.* 2020; RySS 2019).

**Reasons for adoption:** Farmers primarily chose to adopt ZBNF to reduce the cost of cultivation (Bishnoi and Bhati 2017; Biswas 2020; Khadse *et al.* 2018; Khurana and Kumar 2020; Mier y Terán Giménez Cacho *et al.* 2019; Münster 2017). Since ZBNF does not require chemical inputs, overall input costs decrease dramatically, thereby reducing the need for credit to purchase chemical inputs. One farmer stated that costs were so low that he was no longer concerned with achieving a certain yield to return a profit (Bishnoi and Bhati 2017). Khurana and Kumar (2020) conducted focus groups with 142 farmers across five districts and found that 100 percent of farmers surveyed felt their costs decreased under ZBNF (Khurana and Kumar 2020). In a concurrent survey of 40 farmers, 90 percent of farmers believed costs decreased under ZBNF, and 10 percent felt that costs remained the same as in conventional farming (Khurana and Kumar 2020). In the Prakasam district

of Andhra Pradesh, farmers held diverging views on the cost impacts of ZBNF (Sarada and Kumar 2018). Forty-five percent of farmers surveyed in Prakasam believed that ZBNF did not reduce costs relative to conventional farming, and 42 percent believed ZBNF did reduce costs. The remaining 13 percent were undecided (Sarada and Kumar 2018). The results in Prakasam were likely different from the aforementioned studies due to changes in labor costs. In Karnataka, farmers primarily adopted ZBNF due to health benefits (presumably from reduced exposure to chemicals), followed by food self-sufficiency, environmental reasons, and reduced costs and debt (Khadse *et al.* 2018). Other motivations for adopting ZBNF in Karnataka included economic independence from corporations and spiritual reasons (Khadse *et al.* 2018).

**Barriers to adoption:** Research has shown that barriers to ZBNF adoption include time and labor constraints, access to cows, land access, and tenant farmer arrangements (Bhattacharya 2017; Galab *et al.* 2019a; Khurana and Kumar 2020). Small farmers noted that ZBNF input preparation is time and labor intensive (Galab *et al.* 2019a; Gupta *et al.* 2020; Khurana and Kumar 2020; Reddy *et al.* 2019). Gupta *et al.* (2020) concluded beejamrutham required the most labor out of all ZBNF inputs due to manual seed coating and hand-mixing of ingredients (Gupta *et al.* 2020). The Centre for Study of Science, Technology, and Policy (CSTEP) surveyed 120 farmers and reported that each acre of paddy under ZBNF required 60 hours of work from men, 800 hours from women, and 10 hours of bullock labor, compared to 110 hours of work from men, 530 hours from women, and 40 hours of bullock labor per non-ZBNF acre (CSTEP 2020). Studies found that large farmers were less likely to adopt ZBNF due to labor and time constraints, as the increased costs to hire labor affected their profitability (Das 2020; Gupta *et al.* 2020; Khurana and Kumar 2020). One author claimed ZBNF was not profitable for farmers owning more than five acres (Das 2020). Although ZBNF's farmer-tofarmer network helps to fill knowledge gaps on implementation, knowledge about how to prepare and use bio-pesticides (e.g., neemstra, agniastra, brahmastra) is still a constraint to adoption (Galab *et al.* 2019a; Sarada and Kumar 2018).

### Case Study

A College Lecturer turns into a Profitable Natural Farming Farmer- A successful case of Reddy Mahalakshmanudu at Vadlamuru Village, Agiripally Mandal, Krishna District, and Andhra Pradesh State.

**Name:** Mr. Reddy Mahalakshmanudu

**Village:** Vadlamuru

**Mandal:** Agiripally

**District:** Krishna

**Contact No:** 9640727329

**Education:** M.Ed

Mr Reddy Mahalakshmanudu, a college lecturer who turned in a progressive Natural farming farmer.



### Training & Motivation

His father and other villagers were basically farmers practicing conventional method of farming. His family they observed wiping out of their mango orchard due to indiscriminate use of chemical and low income from orchard and even from other crops. In mango, they were facing the problem of stem and leaf falling, no fruit bearing and uneven ripening along with reduced production. He highlighted that the soil condition was deteriorating, as the soil turned harder and soil organisms were disappearing. Mr Reddy Mahalakshmanudu is well educated person and knew the value of soil and had respect towards agriculture made him to meet his friend Srinivas Rao, who was working has a Community Resource Person (CRP) under Rythu Sadhikara Samstha (RySS) in his village. Discussing with his friend he decided to practice natural farming in 2016. The training programme of Sri Subhash Pallekhar motivated him towards natural farming and there he learned all the preparation methods of natural farming inputs like Jeevamrutham, Beejamrutham, Neemastra, Gana Jeevamrutham etc.

### Adoption of natural farming practices and achievements

Initially he started natural farming in his 4 acres of land,

he incurred losses in his early stage but he continued because natural farming had a positive effect on his family health. After 3 years, his field started earning well and then he brought extra 5 acres of land as lease to continue natural farming along with his 4 acres of land. He prepare his input himself and expressed it as nor difficult neither labour intenstive. He prepares beejamritham, ghanajivamritham, jeevamritham, Gokrupa amrutham, mulching, growth regulators (fish amino acid, egg amino acid, botanical extract, saphthanya kashayam, etc.), and kashayams for pest management. Low cost input substitution, like he uses overripped mangoes (which has no market value) instead of jaggery in jeevamritham, use of waste plaste bottle with lure (saves moneys from Rs 10-15 and use of bananas to ripe Managoes evenly). Better use of by products, used in selling and also in preparation of natural farming inputs in his field. His farm serves as a model farm for not only the farmers of his village but also the farmers of nearby villages and officials too.

**Table 1:** Comparison between Natural and Conventional Farming by Mr. Reddy Mahalakshmanudu

Parameters	Natural Farming		Conventional Farming	
	Mango	Paddy	Mango	Paddy
Cost of cultivation (in ₹)	51,000	25,000	74,000	23,000
Production (Quintals)	30	20	15	18
Gross returns (in ₹)	2,40,000	93,500	70,000	28,000
Net returns (in ₹)	1,99,000	68,500	24,000	5,000
B:C ratio	4.71	3.74	0.95	1.2

**Benefits**

He expressed that by practicing natural farming in his farm, he got profits and notable increase in production of crops by 10 per cent then conventional farmers. Enhanced quality of vegetables and improved the shelf life of mango and taste of rice has improved double fold. He is an innovative farmer, as he tries practicing new thing in his farm for better yield and incocme, for example; he tried grafting in a mango tree with 17 varities of mangoes. He sells his mangos and other vegetables to the local officials and school teachers and other public representatives, this has gained good recognition. There no issue regarding marketing, as he does direct marketing of his produce without middlemen to the consumers of cities and towns like Visakapatnam, Vijaywada, Hyderabad, Bengaluru etc (Whatsapp group with his consumers to promote and sell his produce). He rears his indigenious cows naturally, sells their milk at good price and this adds

a amount of money to his income. He is awarded as best farmer from Krishna district by Rythu Nastham Foundation, 2018. Mr Reddy Mahalakshmanudu success story is an inspiration to other, who would like to earn good profits in Agriculture and respect in society. He stated that by providing good market facilities for the natural produce and recognising natural farming farmers will leads to adopt natural farming by all farmers of the state.

**Challenges to adopt ZBNF**

Natural farming is eco-friendly sustainable to the environment maintains good health of soil, plant as well as human beings by increasing beneficial microbial population, chemical-free nutrients supply to plants and toxic-free food supplies to consumers (man and animals). But still, farmers are not going to adapt this technology due to some lacunas so the govt, researchers, scientist and extension workers should think about major challenges to success this zero budget natural farming technology in a large area, and according to authors view some of the major challenges are listed below.

1. The labor requirement is increased compared to conventional farming.
2. The demand for animal manure is high. On a national scale, the number of cattle in India could not support this level of manure application.
3. With the demand and consumption pattern constantly changing when it comes to high-value products.
4. Better technology and high investment are required on farmland. Well developed heavy machinery, implements are not used in natural farming due to it creates soil compaction even there is no use of tractors in it.
5. Weakened agricultural market infrastructure- there is no value of natural products in large scale areas even the price also similar to chemically produced products.
6. No scientific validation- microbial composition, efficiency, and impact of jivamrith, bijamrith, bramhastra, dashaparni kashaya not yet tested and there is no scientific data on it.



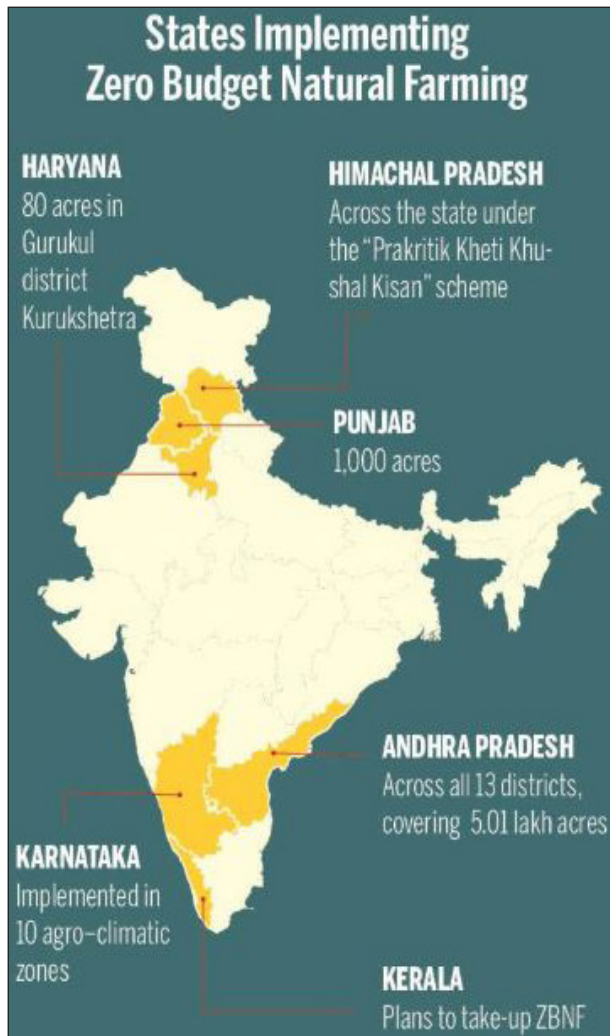
7. Hybrid varieties, not permitted-continuously increasing global population food is scarce to all populations. Even by using chemicals, we are not reaching our food production target, without hybrids, it is impossible to reach the target.
8. Promotion of ZBNF on a large scale without scientific validation and under the political influence- no scientific data on crop yield.
9. Pest management is difficult- different crop-specific weeds, diseases, insects are damaging to crop drastically, by using natural products its control is not satisfactory to farmer's level.
10. Quality planting material and other proven techniques like GMO's are not considered in ZBNF.
11. Criticizes other agroecological and organic farming techniques.
12. Non-availability of indigenous cow, it contains Millions of beneficial microbes and pathogen-free dung.
13. As ZBNF adapted in India by Mr. Subash Palekar is not adopted in his own state Maharashtra.
6. Scheme for cattle and other live stock farmers for the purpose of its maintenance and marketing of live stock produce.
7. Ensure the availability of natural farming inputs in all villages under Rythu Barosa Kendras it will be helpful for initial period farmers to get good yields
8. More training and demonstration respect to crop specific will be given to natural farming farmers and also teach the techniques of preparation of natural farming inputs.
9. Increase the availability of Non Pesticides Management shops in all villages and also give some financial assistance for the managers of NPMs
10. Need to create special marketing channel for the natural farming inputs that will fetch to get more profits to the natural farming farmers. It will also motivate other farmers also to take up natural farming.

### **Suitable Suggestions for Improving Natural Farming in Andhra Pradesh State**

1. Essential to recognise the natural farming farmers and they need special programmes for their welfare and stability.
2. Creating Brand value to the natural farming produce and also location specific brand identity will be needed like geographical Indication in natural farming for the produce.
3. Marketing will be major issue to the natural farming farmers it needs to be resolved most priority basis and special price for the natural farming produce will be ensured.
4. Hand holding support from the government side will be needed to the natural farming farmers in initial periods.
5. The state department of agriculture and other related institutes of agriculture like SAUs and KVKs, ATMA, DAATTC, will focus more on natural farming involvement will be needed.

### **CONCLUSION**

Zero budget farming is environmentally friendly. Savings on the cost of seeds, fertilizers, and plant protection chemicals have been substantial. Because of continuous retention of crop residues replenishment the soil fertility, it helps to maintain the soil health. Other thing is that management of pest and diseases is a key component in zero budget natural farming crop production systems. Successfully control pests in ZBNF, it is essential to understand the interactions of different components in a specific ecosystem. The new system of farming has free debt trap of farmers and it has instilled in them a renewed sense of confidence to make farming an economically viable venture. The challenges and opportunities are two parameters that show the systems lacunas to researchers, scientists, and extension workers and benefits to adopters, and policy intervention is necessary to make the success.



Source: Answer to a question in Lok Sabha

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# Influence of Preservatives and Biodegradable Nano Silver Film on Post-harvest Life of *Jasminum sambac* Cv. “Mysuru Mallige”

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## ABSTRACT

Experiment was carried out in the Department of Floriculture and Landscape Architecture, College of Horticulture Mysuru during 2019-2021 to find out the effect of chemical preservatives and biodegradable nano silver film on post-harvest life of *Jasminum sambac* cv. Mysuru Mallige. The experiment was laid out in Factorial Completely randomized Design (FCRD) with 15 treatment and two replications. The periodical observations recorded every 12 hrs from 0 hrs to 36 hrs. were physiological loss of weight, freshness index and shelf-life. Results of this experiment envisaged that flowers which are treated with 5 per cent boric acid and packed in 60 micron biodegradable nano silver film recorded less physiological loss of weight with maximum fragrance index and shelf-life of the flower.

## HIGHLIGHTS

- Jasmine (*Jasminum spp.*) is one of the most fragrant flowers belongs to the family Oleaceae and flowers have a great economic value in India and mainly used as loose flower for making garland and oil extraction. Among the commercial species of jasmine, *Jasminum sambac* Ait. commonly known as Arabian jasmine, Tuscan jasmine, Mogra and Bela. There have several cultivars like Motia, Double Mogra, Single Mogra, Gundu Mallige, Bela, Khoya, Madanbana, Ramabana and Mysuru Mallige. Among these cultivars, Mysuru Mallige (Mysuru Jasmine) being associated with the city of Mysuru, patronized by the wodeyar of the kingdom of Mysuru because of its flower fragrance. The Mysuru Jasmine has got Geographical Indication status (GI under Registration of Protection Act 1999) under agriculture commodity (69<sup>th</sup> G.I. Product of India) by Govt. of India during 2007-08 for its unique fragrance and flowering characters and commonly cultivated in Mysore and Mandya regions of Karnataka.

**Keywords:** Jasmine, Preservatives, Nano silver film, Shelf-life

Mysuru Mallige flowers are highly perishable in nature and got good demand in local market as well as outside market like Kerala, Tamilnadu, Mumbai etc. One of the major problems faced by grower are lack of suitable packaging technology for export. The flowers are very delicate and show signs of wilting with abrupt loss of fragrance within 24 -36 hours after harvest. Under normal condition, jasmine flowers do not retain for more than a day and show

a sign of browning or rotting of petals on the second day of harvest with an abrupt loss in fragrance. Krishnamoorthy (1990) reported that packaging is a fundamental tool for post-harvest management

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of highly perishable commodities and adequate packaging protects the produce from physical, physiological and pathological deterioration during transport and marketing, enhancing their shelf-life by retaining their attractiveness. So, standardization of post-harvest techniques to extend shelf-life of Mysuru Mallige flowers will be helpful to farmers to exploit distant market. Keeping this in mind, a study was undertaken to enhance the post-harvest life of Mysuru Mallige.

## MATERIALS AND METHODS

The experiment was carried out in the Department of Floriculture and Landscape Architecture, College of Horticulture Mysuru during 2019-2021 to find out the effect of chemical preservatives and biodegradable nano silver film on post-harvest life of *Jasminum sambac* cv. Mysuru Mallige. The experiment was laid out in Factorial Completely randomized Design (FCRD) with two replications and two factors: Different preservatives at different concentration and different thickness of biodegradable nano silver film. The first factor comprised of five treatments viz., T<sub>1</sub>: Boric acid at 4 per cent, T<sub>2</sub>: Boric acid at 5 per cent, T<sub>3</sub>: Silver nitrate at 0.2 per cent, T<sub>4</sub>: Silver nitrate at 0.3 per cent and T<sub>5</sub>: Water spray (Control) and second factor contains 3 treatments viz., B<sub>1</sub>: 40-micron Nano silver film, B<sub>2</sub>: 60-micron Nano silver film and B<sub>3</sub>: 70-micron Nano silver film. 500g of uniform size, freshly harvested flowers buds are used for each treatment and observation like physiological losses of weight (PLW), freshness score and shelf-life were recorded during storage of flowers at 24 hours of intervals.

**Table 1:** Freshness score details

Freshness index	Score
Original colour/ fresh flower	1
Partial fading of original colour	2
Complete fading of original colour	3
1 to 10% brown	4
11 to 15% brown	5
16 to 30% brown	6
30 to 50% brown	7
51 to 75% brown	8
76 to 90% brown	9
All brown	10

Physiological losses of weight was calculated by subtracting fresh weight of flowers at the time of

packing and at 12<sup>th</sup>, 24<sup>th</sup>, and 36<sup>th</sup> hr after harvesting of flowers and expressed as percentage. Freshness score were recorded based on scale, the score was expressed on 1 to 10 (Isac, 2015) and average was calculated. Shelf-life of the flower was measured as time taken to wilt 50% of flowers. The resulted data were statistically analyzed using the method of Panase and Sukhatme (1967).

## RESULTS AND DISCUSSION

The result obtaining with respect to Physiological loss in weight, Freshness index and Shelf life of Mysuru Mallige flowers are presented in the table 2, 3 and 4 respectively.

### Physiological loss in weight of the flowers (%)

The data on physiological loss in weight of the flower (%) as influenced by different preservatives and biodegradable packaging materials and their interaction effects are presented in Table 2.

### Chemical preservatives (T)

Among different chemical preservatives, the minimum physiological loss in weight of the flowers (1.02 per cent, 1.87 per cent and 2.52 per cent) was recorded in 5 per cent boric acid (T<sub>2</sub>) and maximum physiological loss in weight of the flowers (4.50 per cent, 6.31 per cent and 7.26 per cent) was recorded in T<sub>5</sub> i.e., water spray at 12, 24 and 36 hours after imposing the treatments respectively during 2019-20. Similarly, during 2020-21, the minimum physiological loss in weight of the flowers (0.94 per cent, 1.83 per cent and 2.45 per cent) was recorded in T<sub>2</sub> i.e., spray of 5 per cent boric acid and maximum physiological loss in weight of the flowers (4.45 per cent, 6.26 per cent and 7.24 per cent) was recorded in water spray (T<sub>5</sub>, control) at 12, 24 and 36 hours after imposing the treatment respectively. Pooled data revealed that, spray of 5 per cent boric acid (T<sub>2</sub>) recorded minimum physiological loss in weight (0.98 ± 0.06 per cent, 1.85 ± 0.03 per cent and 2.49 ± 0.05 per cent) and maximum physiological loss in weight of the flower (4.47 ± 0.04 per cent, 6.29 ± 0.04 per cent and 7.25 ± 0.01 per cent) was recorded in T<sub>5</sub> i.e., water spray at 12, 24 and 36 hours after imposing the treatment respectively.

### Biodegradable packaging material (B)

Among the different thickness of biodegradable

**Table 2:** Effect of different preservatives and biodegradable packaging material on physiological loss in weight (%)

Treatments	2019-20			2020-21			Pooled Mean		
	12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.
<b>Preservatives (T)</b>									
T <sub>1</sub>	1.29	2.09	2.99	1.27	2.07	2.94	1.28 ± 0.01	2.08 ± 0.02	2.97 ± 0.03
T <sub>2</sub>	1.02	1.87	2.52	0.94	1.83	2.45	0.98 ± 0.06	1.85 ± 0.03	2.49 ± 0.05
T <sub>3</sub>	3.22	4.50	5.58	3.16	4.46	5.55	3.19 ± 0.04	4.48 ± 0.02	5.57 ± 0.03
T <sub>4</sub>	2.87	4.23	4.85	2.76	4.17	4.78	2.81 ± 0.08	4.20 ± 0.04	4.81 ± 0.05
T <sub>5</sub>	4.50	6.31	7.26	4.45	6.26	7.24	4.47 ± 0.04	6.29 ± 0.04	7.25 ± 0.01
S. Em. ±	0.05	0.04	0.04	0.05	0.04	0.04	—	—	—
C.D@5%	0.13	0.12	0.12	0.13	0.12	0.13	—	—	—
<b>Biodegradable packaging material (B)</b>									
B <sub>1</sub>	2.84	4.13	5.01	2.73	4.11	4.96	2.79 ± 0.08	4.12 ± 0.01	4.99 ± 0.03
B <sub>2</sub>	2.34	3.45	4.29	2.30	3.43	4.24	2.32 ± 0.02	3.44 ± 0.02	4.27 ± 0.03
B <sub>3</sub>	2.57	3.82	4.62	2.51	3.74	4.57	2.54 ± 0.04	3.78 ± 0.06	4.60 ± 0.04
S. Em. ±	0.04	0.03	0.03	0.04	0.03	0.03	—	—	—
C.D@5%	0.10	0.09	0.09	0.10	0.09	0.10	—	—	—
<b>Interactions</b>									
T <sub>1</sub> B <sub>1</sub>	1.36	2.28	3.24	1.35	2.23	3.17	1.36 ± 0.01	2.26 ± 0.04	3.20 ± 0.05
T <sub>1</sub> B <sub>2</sub>	1.21	1.92	2.80	1.21	1.93	2.77	1.21 ± 0.00	1.93 ± 0.01	2.78 ± 0.02
T <sub>1</sub> B <sub>3</sub>	1.31	2.08	2.94	1.26	2.04	2.90	1.29 ± 0.03	2.06 ± 0.02	2.92 ± 0.03
T <sub>2</sub> B <sub>1</sub>	1.39	2.11	2.92	1.27	2.07	2.87	1.33 ± 0.09	2.09 ± 0.03	2.90 ± 0.03
T <sub>2</sub> B <sub>2</sub>	0.75	1.65	2.23	0.68	1.62	2.17	0.72 ± 0.05	1.63 ± 0.02	2.20 ± 0.05
T <sub>2</sub> B <sub>3</sub>	0.93	1.83	2.42	0.87	1.80	2.32	0.90 ± 0.04	1.82 ± 0.02	2.37 ± 0.07
T <sub>3</sub> B <sub>1</sub>	3.38	4.81	5.90	3.27	4.80	5.87	3.33 ± 0.08	4.80 ± 0.00	5.88 ± 0.02
T <sub>3</sub> B <sub>2</sub>	3.06	4.03	5.21	3.03	4.03	5.17	3.05 ± 0.02	4.03 ± 0.00	5.19 ± 0.03
T <sub>3</sub> B <sub>3</sub>	3.21	4.65	5.64	3.17	4.55	5.60	3.19 ± 0.03	4.60 ± 0.07	5.62 ± 0.03
T <sub>4</sub> B <sub>1</sub>	3.16	4.55	5.27	2.97	4.50	5.22	3.07 ± 0.14	4.53 ± 0.04	5.24 ± 0.04
T <sub>4</sub> B <sub>2</sub>	2.53	3.95	4.35	2.50	3.88	4.28	2.51 ± 0.02	3.92 ± 0.05	4.32 ± 0.05
T <sub>4</sub> B <sub>3</sub>	2.92	4.20	4.92	2.80	4.13	4.83	2.86 ± 0.08	4.17 ± 0.05	4.88 ± 0.06
T <sub>5</sub> B <sub>1</sub>	4.91	6.90	7.72	4.80	6.95	7.68	4.85 ± 0.08	6.93 ± 0.04	7.70 ± 0.02
T <sub>5</sub> B <sub>2</sub>	4.14	5.72	6.86	4.10	5.67	6.83	4.12 ± 0.03	5.69 ± 0.04	6.85 ± 0.02
T <sub>5</sub> B <sub>3</sub>	4.46	6.32	7.20	4.44	6.17	7.20	4.45 ± 0.01	6.24 ± 0.11	7.20 ± 0.00
S. Em. ±	0.08	0.07	0.07	0.08	0.07	0.08	—	—	—
C.D@5%	0.23	0.20	0.20	0.23	0.21	0.22	—	—	—

T<sub>1</sub>: Boric acid at 4 per cent; T<sub>2</sub>: Boric acid at 5 per cent; T<sub>3</sub>: Silver nitrate at 0.2 per cent; T<sub>4</sub>: Silver nitrate at 0.3 per cent; T<sub>5</sub>: Water spray; B<sub>1</sub>: 40-micron Nano silver film; B<sub>2</sub>: 60-micron Nano silver film; B<sub>3</sub>: 70-micron Nano silver film.

packaging material, flowers which are packed in 60 micron nano silver film (B<sub>2</sub>) recorded minimum physiological loss in weight (2.34 per cent, 3.45 per cent and 4.29 per cent) and maximum physiological loss in weight (2.84 per cent, 4.13 per cent and 5.01 per cent) of the flower was recorded in 40 micron nano silver film (B<sub>1</sub>) at 12, 24 and 36 hours after imposing the treatment respectively during 2019-20. Similarly, during 2020-21, the flowers which were packed in 60 micron nano silver film (B<sub>2</sub>) recorded minimum physiological loss in weight (2.30 per cent, 3.43 per cent and 4.24 per cent). Maximum physiological loss in weight (2.73 per cent, 4.11 per cent and 4.96 per cent) was recorded in B<sub>1</sub> i.e.,

40 micron nano silver film at 12, 24 and 36 hours after imposing the treatment respectively. Pooled data revealed that, minimum physiological loss in weight of the flower (2.32 ± 0.02 per cent, 3.44 ± 0.02 per cent and 4.27 ± 0.03 per cent) was recorded in B<sub>2</sub> (60 micron biodegradable nano silver film) whereas maximum physiological loss in weight of the flower (2.79 ± 0.08, 4.12 ± 0.01, and 4.99 ± 0.03 per cent) was recorded in 40-micron biodegradable nano silver film (B<sub>1</sub>).

### Interaction (T × B)

Interaction effect was significant on physiological loss in weight (%) at 12, 24 and 36 hours after



imposing the treatment in both the season *i.e.*, 2019-20 and 2020-21.

The interaction results showed that, T<sub>2</sub>B<sub>2</sub> (spray of 5 per cent boric acid and packed in 60 micron biodegradable nano silver film) recorded minimum physiological loss in weight of the flower (0.75 per cent, 1.65 per cent and 2.23 per cent) followed by T<sub>2</sub>B<sub>3</sub> *i.e.*, 0.93 per cent, 1.83 per cent and 2.42 per cent. Maximum physiological loss in weight (4.91 per cent, 6.90 per cent and 7.72 per cent) was recorded in T<sub>5</sub>B<sub>1</sub> interaction (water spray and flowers are packed in 40 micron biodegradable nano silver film) at 12, 24 and 36 hours after imposing the treatment respectively during 2019-20. During 2020-21, flowers which are treated with 5 per cent boric acid and packed in 60 micron biodegradable nano silver film (T<sub>2</sub>B<sub>2</sub>) recorded minimum physiological loss in weight (0.68 per cent, 1.62 per cent and 2.17 per cent) followed by T<sub>2</sub>B<sub>3</sub> (0.87 per cent, 1.80 per cent and 2.32 per cent). Interaction of T<sub>5</sub>B<sub>1</sub> (water spray and storage in 40 micron biodegradable nano silver film) recorded maximum physiological loss in weight of the flower (4.80 per cent, 6.95 per cent and 7.68 per cent) at 12, 24 and 36 hours after imposing the treatment respectively. Pooled mean revealed that, T<sub>2</sub>B<sub>2</sub> (Spray of 5 per cent boric acid and packed in 60 micron biodegradable nano silver film) interaction recorded minimum physiological loss in weight (0.72 ± 0.05 per cent, 1.63 ± 0.02 per cent and 2.20 ± 0.05 per cent) and maximum physiological loss in weight (4.85 ± 0.08 per cent, 6.93 ± 0.04 per cent and 7.70 ± 0.02 per cent) was recorded in T<sub>5</sub>B<sub>1</sub> interaction (water spray and flowers are packed at 40 micron biodegradable nano silver film).

Boric acid is an anti-senescence agent, increases water balance in flower, delay the ethylene production, minimizes the physiological loss of weight and phenol accumulation. Similar results were also obtained by Jawaharlal *et al.* (2012) and Mukhopadhyay *et al.* (1980) in jasmine. Nano silver film has most effective bactericidal property against a wide range of pathogenic microorganism, including bacteria, yeasts, fungi and viruses (Martinez *et al.* 2012) Thus, it also increases shelf-life of flower without altering physical characteristics (Emamifar *et al.* 2010).

### Freshness Index (10 point scale)

The data on freshness index as influenced by

different chemical preservatives and biodegradable packaging material and their interaction effects during 2019-20 and 2020-21 are presented in Table 3.

### Chemical preservatives (T)

The treatment consisting of boric acid spray at 5 per cent (T<sub>2</sub>) recorded maximum freshness index score of 9.52, 8.33 and 4.78. Whereas minimum freshness index (8.00, 5.60 and 2.09) was recorded in T<sub>5</sub> (Water spray) at 12, 24 and 36 hours after imposing the treatment respectively during 2019-20. Among the different spray of chemical preservatives, the maximum freshness index score (9.56, 8.37 and 4.82) was recorded in spray of 5 per cent boric acid (T<sub>2</sub>) and minimum freshness score (8.03, 5.54 and 2.04) was recorded in T<sub>5</sub> *i.e.*, water spray at 12, 24 and 36 hours after imposing the treatment respectively during 2020-21. Pooled data revealed that, spray of 5 per cent boric acid (T<sub>2</sub>) recorded maximum freshness index score (9.54 ± 0.03, 8.35 ± 0.03 and 4.80 ± 0.03) and minimum freshness score (8.02 ± 0.02, 5.57 ± 0.04 and 2.07 ± 0.04) was recorded in T<sub>5</sub> *i.e.*, water spray at 12, 24 and 36 hours after imposing the treatment respectively.

### Biodegradable packaging material (B)

Flowers packed in 60 micron biodegradable nano silver film (B<sub>2</sub>) recorded maximum freshness index score (9.01, 7.40 and 3.95), whereas 40 micron biodegradable nano silver film (B<sub>1</sub>) recorded minimum freshness index (8.74, 6.55 and 2.85) at 12, 24 and 36 hours after imposing the treatment respectively during 2019-20. Among the different thickness of biodegradable packaging material, flowers which are packed in 60 micron nano silver film (B<sub>2</sub>) recorded maximum freshness index score of 9.03, 7.40 and 3.96. Minimum freshness index score (8.75, 6.56 and 2.86) was recorded in 40 micron nano silver film (B<sub>1</sub>) at 12, 24 and 36 hours after imposing the treatment respectively during 2020-21. Pooled data revealed that, B<sub>2</sub> *i.e.*, flowers packed in 60 micron biodegradable nano silver film recorded maximum freshness index score (9.02 ± 0.01, 7.40 ± 0.00 and 3.96 ± 0.01) and minimum freshness index score (8.75 ± 0.01, 6.56 ± 0.01 and 2.86 ± 0.01) was recorded in 40 micron nano silver film (B<sub>1</sub>) at 12, 24 and 36 hours after imposing the treatment respectively.

**Table 3:** Effect of different preservatives and biodegradable packaging material on freshness index (10 point scale)

Treatments	2019-20			2020-21			Pooled mean		
	12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.	12 hrs.	24 hrs.	36 hrs.
<b>Preservatives (P)</b>									
T <sub>1</sub>	9.32	7.62	4.06	9.32	7.66	4.10	9.32 ± 0.00	7.64 ± 0.03	4.08 ± 0.03
T <sub>2</sub>	9.52	8.33	4.78	9.56	8.37	4.82	9.54 ± 0.03	8.35 ± 0.03	4.80 ± 0.03
T <sub>3</sub>	8.58	6.23	2.63	8.58	6.20	2.58	8.58 ± 0.00	6.22 ± 0.02	2.61 ± 0.04
T <sub>4</sub>	9.02	7.11	3.43	9.02	7.16	3.47	9.02 ± 0.00	7.14 ± 0.04	3.45 ± 0.03
T <sub>5</sub>	8.00	5.60	2.09	8.03	5.54	2.04	8.02 ± 0.02	5.57 ± 0.04	2.07 ± 0.04
S. Em. ±	0.03	0.07	0.05	0.03	0.07	0.05	—	—	—
C.D@5%	0.09	0.19	0.15	0.08	0.20	0.14	—	—	—
<b>Biodegradable packaging material (B)</b>									
B <sub>1</sub>	8.74	6.55	2.85	8.75	6.56	2.86	8.75 ± 0.01	6.56 ± 0.01	2.86 ± 0.01
B <sub>2</sub>	9.01	7.40	3.95	9.03	7.40	3.96	9.02 ± 0.01	7.40 ± 0.00	3.96 ± 0.01
B <sub>3</sub>	8.91	6.99	3.39	8.93	6.99	3.40	8.92 ± 0.01	6.99 ± 0.00	3.40 ± 0.01
S. Em. ±	0.02	0.05	0.04	0.02	0.05	0.04	—	—	—
C.D@5%	0.07	0.15	0.12	0.06	0.15	0.11	—	—	—
<b>Interactions</b>									
T <sub>1</sub> B <sub>1</sub>	9.27	7.30	3.50	9.27	7.33	3.55	9.27 ± 0.00	7.32 ± 0.02	3.53 ± 0.04
T <sub>1</sub> B <sub>2</sub>	9.40	7.90	4.60	9.40	7.93	4.65	9.40 ± 0.00	7.92 ± 0.02	4.63 ± 0.04
T <sub>1</sub> B <sub>3</sub>	9.30	7.67	4.07	9.30	7.70	4.10	9.30 ± 0.00	7.69 ± 0.02	4.09 ± 0.02
T <sub>2</sub> B <sub>1</sub>	9.33	7.90	4.53	9.37	7.95	4.57	9.35 ± 0.03	7.93 ± 0.04	4.55 ± 0.03
T <sub>2</sub> B <sub>2</sub>	9.67	8.70	5.00	9.70	8.73	5.05	9.69 ± 0.02	8.72 ± 0.02	5.03 ± 0.04
T <sub>2</sub> B <sub>3</sub>	9.57	8.40	4.80	9.60	8.43	4.85	9.59 ± 0.02	8.42 ± 0.02	4.83 ± 0.04
T <sub>3</sub> B <sub>1</sub>	8.53	5.83	2.10	8.53	5.80	2.05	8.53 ± 0.00	5.82 ± 0.02	2.08 ± 0.04
T <sub>3</sub> B <sub>2</sub>	8.63	6.83	3.30	8.63	6.80	3.25	8.63 ± 0.00	6.82 ± 0.02	3.28 ± 0.04
T <sub>3</sub> B <sub>3</sub>	8.57	6.03	2.50	8.57	6.00	2.45	8.57 ± 0.00	6.02 ± 0.02	2.48 ± 0.04
T <sub>4</sub> B <sub>1</sub>	8.80	6.40	2.63	8.80	6.45	2.67	8.80 ± 0.00	6.43 ± 0.04	2.65 ± 0.03
T <sub>4</sub> B <sub>2</sub>	9.20	7.60	4.27	9.20	7.65	4.30	9.20 ± 0.00	7.63 ± 0.04	4.29 ± 0.02
T <sub>4</sub> B <sub>3</sub>	9.07	7.33	3.40	9.07	7.37	3.45	9.07 ± 0.00	7.35 ± 0.03	3.43 ± 0.04
T <sub>5</sub> B <sub>1</sub>	7.77	5.30	1.50	7.80	5.25	1.45	7.79 ± 0.02	5.28 ± 0.04	1.48 ± 0.04
T <sub>5</sub> B <sub>2</sub>	8.17	5.97	2.60	8.20	5.90	2.55	8.19 ± 0.02	5.94 ± 0.05	2.58 ± 0.04
T <sub>5</sub> B <sub>3</sub>	8.07	5.53	2.17	8.10	5.47	2.13	8.09 ± 0.02	5.50 ± 0.04	2.15 ± 0.03
S. Em. ±	0.05	0.12	0.09	0.05	0.12	0.08	—	—	—
C.D@5%	0.15	0.34	0.27	0.14	0.34	0.24	—	—	—

T<sub>1</sub>: Boric acid at 4 per cent; T<sub>2</sub>: Boric acid at 5 per cent; T<sub>3</sub>: Silver nitrate at 0.2 per cent; T<sub>4</sub>: Silver nitrate at 0.3 per cent; T<sub>5</sub>: Water spray; B<sub>1</sub>: 40-micron Nano silver film; B<sub>2</sub>: 60-micron Nano silver film; B<sub>3</sub>: 70-micron Nano silver film.

### Interaction (T × B)

The interaction results noted that, T<sub>2</sub>B<sub>2</sub> (Spray of 5 per cent boric acid and packed in 60 micron biodegradable nano silver film) interaction recorded maximum freshness index score (9.67, 8.70 and 5.00) followed by the treatment includes spray of 5 per cent boric acid and packed in 70 micron biodegradable nano silver film (T<sub>2</sub>B<sub>3</sub>) i.e., 9.57, 8.40 and 4.80. Minimum freshness index of 7.77, 5.30 and 1.50 was recorded in T<sub>5</sub>B<sub>1</sub> interaction (Water spray + flowers are packed in 40 micron biodegradable nano silver film) at 12, 24 and 36 hours after imposing the treatment respectively during 2019-20. During 2020-21, flowers which are treated with spray of 5% boric

acid and packed in 60 micron biodegradable nano silver film (T<sub>2</sub>B<sub>2</sub>) recorded maximum freshness index score of 9.70, 8.73 and 5.05 followed by T<sub>2</sub>B<sub>3</sub> (Spray of 5 per cent boric acid and packed in 70 micron biodegradable nano silver film) (9.60, 8.43 and 4.85). Interaction of T<sub>5</sub>B<sub>1</sub> (water spray and flowers are packed in 40 micron biodegradable nano silver film) recorded minimum freshness index score of 7.80, 5.25 and 1.45 at 12, 24 and 36 hours after imposing the treatment respectively. Pooled mean revealed that, T<sub>2</sub>B<sub>2</sub> (spray of 5% boric acid and packed in 60 micron biodegradable nano silver film) interaction recorded maximum freshness index score (9.69 ± 0.02, 8.72 ± 0.02 and 4.83 ± 0.04) and minimum freshness index score (7.79 ± 0.02, 5.28 ± 0.04 and



1.48 ± 0.04) was recorded in flowers are sprayed with water and packed in 40 micron biodegradable nano silver film (T<sub>5</sub>B<sub>1</sub>) interaction at 12, 24 and 36 hours after imposing the treatment respectively.

Boric acid proved effective by registering higher levels of moisture, relative water content, lowest rates of PLW. This, in turn reduces solute leakage from flowers, indicating increased membrane integrity of flowers. All these factors proved effective in retaining freshness index of flowers (Jawaharlal *et al.*, 2012 in jasmine). Similar results were also obtained by Maruthamuthuchandran *et al.*, (2018) and Mukhopadhyay *et al.*, (1980) in jasmine. Flowers packed in 60 micron nano silver film are capable of modifying the atmosphere in the packs and thus allowing the flowers to be stored for long hours without affecting the freshness (Tavakoli *et al.* 2017). These results are in close agreement with the findings of Madaiah and Reddy (1994) and Mukhopadhyay *et al.*, (1980) in jasmine.

### Shelf-life (hrs)

The data on shelf-life (hrs) of the flowers as influenced by different spray chemical preservatives and biodegradable packaging material and their interaction are presented in Table 4.

### Chemical preservatives (T)

Among the different spray of chemical preservatives, the maximum shelf-life of the flower (43.05 hrs) was recorded in T<sub>2</sub> (Spray of 5 per cent boric acid) and minimum shelf-life of the flower (33.59 hrs) was recorded in T<sub>5</sub> i.e., flowers were treated with only water spray during 2019-20. During 2020-21, the maximum shelf-life of the flower (43.14 hrs) was recorded in treatment where flowers were treated with 5 per cent boric acid and minimum shelf-life (34.07 hrs) was recorded in water spray (T<sub>5</sub>- control). Pooled data revealed that, spray of 5 per cent boric acid (T<sub>2</sub>) recorded maximum shelf-life (43.09 ± 0.06 hrs) and minimum shelf-life (34.04 ± 0.06 hrs) was recorded in T<sub>5</sub> i.e., water spray.

### Biodegradable packaging material (B)

Among the different thickness of biodegradable packaging material, flowers which are packed in 60 micron nano silver film (B<sub>2</sub>) was recorded maximum shelf-life (40.08 hrs) and minimum shelf-

life (38.27 hrs) was recorded in 40 micron nano silver film (B<sub>1</sub>) during 2019-20. During 2020-21, the flowers which are packed in 60 micron nano silver film (B<sub>2</sub>) recorded maximum shelf-life of the flower (40.20 hrs) whereas minimum shelf-life of the flower (38.31 hrs) was recorded in B<sub>1</sub> i.e., 40 micron nano silver film. Pooled mean data indicated that, maximum shelf-life of the flower (40.14 ± 0.08 hrs) was recorded in B<sub>2</sub> (60 micron biodegradable nano silver film) whereas minimum shelf-life of the flower (38.29 ± 0.03 hrs) was recorded in 40 micron biodegradable nano silver film (B<sub>1</sub>).

**Table 4:** Effect of different preservatives and biodegradable packaging material on shelf-life (hrs)

Treatments	2019-20	2020-21	Mean
<b>Preservatives</b>			
T <sub>1</sub>	42.35	42.04	42.20 ± 0.07
T <sub>2</sub>	43.05	43.14	43.09 ± 0.06
T <sub>3</sub>	37.37	37.33	37.35 ± 0.02
T <sub>4</sub>	39.55	40.08	40.02 ± 0.09
T <sub>5</sub>	33.59	34.07	34.04 ± 0.06
S. Em. ±	0.14	0.15	
C.D@5%	0.40	0.44	
<b>Biodegradable packaging material</b>			
B <sub>1</sub>	38.27	38.31	38.29 ± 0.03
B <sub>2</sub>	40.08	40.20	40.14 ± 0.08
B <sub>3</sub>	39.11	39.16	39.14 ± 0.04
S. Em. ±	0.11	0.12	—
C.D@5%	0.31	0.34	—
<b>Interactions</b>			
T <sub>1</sub> B <sub>1</sub>	41.25	41.42	41.33 ± 0.12
T <sub>1</sub> B <sub>2</sub>	42.50	42.58	42.54 ± 0.06
T <sub>1</sub> B <sub>3</sub>	42.10	42.13	42.12 ± 0.02
T <sub>2</sub> B <sub>1</sub>	41.50	41.58	41.54 ± 0.06
T <sub>2</sub> B <sub>2</sub>	44.15	44.28	44.22 ± 0.09
T <sub>2</sub> B <sub>3</sub>	43.50	43.55	43.53 ± 0.04
T <sub>3</sub> B <sub>1</sub>	36.45	36.20	36.33 ± 0.04
T <sub>3</sub> B <sub>2</sub>	37.40	37.48	37.44 ± 0.18
T <sub>3</sub> B <sub>3</sub>	37.05	37.12	37.08 ± 0.06
T <sub>4</sub> B <sub>1</sub>	39.00	39.13	39.07 ± 0.05
T <sub>4</sub> B <sub>2</sub>	40.20	40.40	40.30 ± 0.09
T <sub>4</sub> B <sub>3</sub>	39.45	39.50	39.48 ± 0.14
T <sub>5</sub> B <sub>1</sub>	33.15	33.23	33.19 ± 0.06
T <sub>5</sub> B <sub>2</sub>	34.18	34.27	34.23 ± 0.06
T <sub>5</sub> B <sub>3</sub>	33.45	33.52	33.48 ± 0.05
S. Em. ±	0.24	0.26	—
C.D@5%	0.70	0.76	—

T<sub>1</sub>: Boric acid at 4 per cent; T<sub>2</sub>: Boric acid at 5 per cent; T<sub>3</sub>: Silver nitrate at 0.2 per cent; T<sub>4</sub>: Silver nitrate at 0.3 per cent; T<sub>5</sub>: Water spray; B<sub>1</sub>: 40-micron Nano silver film; B<sub>2</sub>: 60-micron Nano silver film; B<sub>3</sub>: 70-micron Nano silver film.

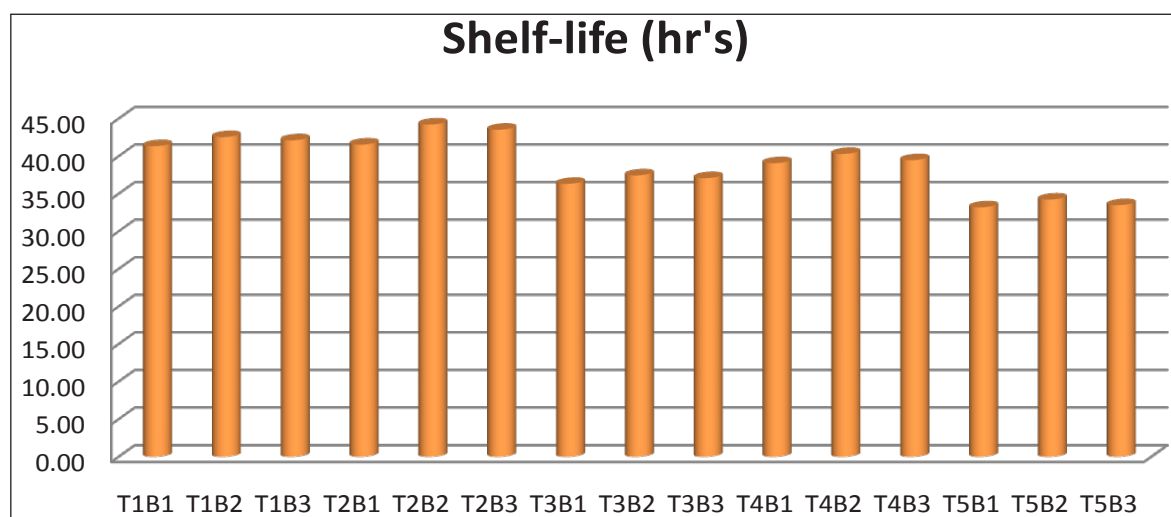


Fig. 1: Effect of different preservatives and biodegradable packaging material on shelf-life (hrs)

### Interaction (T × B)

Interaction effect was significantly influenced on shelf-life of the flowers in both the season *i.e.*, 2019-20 and 2020-21.

The interaction results shows that, T<sub>2</sub>B<sub>2</sub> (spray of 5 per cent boric acid and packed in 60 micron biodegradable nano silver film) interaction recorded maximum shelf-life of the flower (44.15 hrs) followed by T<sub>2</sub>B<sub>3</sub> *i.e.*, (43.50 hrs) and minimum shelf-life of the flower (33.15 hrs) was recorded in T<sub>5</sub>B<sub>1</sub> interaction (water spray + flowers are packed in 40 micron biodegradable nano silver film) during 2019-20. During 2020-21, the flowers which are treated with 5 per cent boric acid and packed in 60 micron biodegradable nano silver film (T<sub>2</sub>B<sub>2</sub>) recorded maximum shelf-life (44.28 hrs) followed by T<sub>2</sub>B<sub>3</sub> (43.55 hrs). Interaction of T<sub>5</sub>B<sub>1</sub> (water spray + flowers are packed in 40 micron biodegradable nano silver film) was recorded with minimum shelf-life of the flower (33.23 hrs). Pooled mean shows that, flowers sprayed with 5 per cent boric acid and stored in 60 micron biodegradable nano silver film (T<sub>2</sub>B<sub>2</sub>) recorded with highest shelf-life (44.22 ± 0.09 hrs) and lowest shelf-life of the flower (33.19 ± 0.06 hrs) was recorded in T<sub>5</sub>B<sub>1</sub> interaction (water spray and flowers are packed at 40 micron biodegradable nano silver film).

Boric acid act as anti-oxidant, improving water balance, delayed ethylene production during storage of flowers (Karuppaiah *et al.* 2006 in jasmine). Similar results were also obtained by Yathindra *et al.* (2018) in jasmine. Maintenance of

optimum humidity and proper balance of CO<sub>2</sub> and O<sub>2</sub> concentration which interns slows down the process of respiration and evapotranspiration and ultimately reduced the PLW and enhances shelf-life of flower. Nano silver film has potential to minimize the microbial load (Martinez *et al.* 2012). Thus, it also increases shelf-life of flower without altering physical characteristics (Emamifar *et al.*, 2010). Boric acid also improves water balance, three-to-six-fold increase in activity of peroxidase and catalase, maximum accumulation of carbohydrates and retaining freshness index and shelf-life of flower by delaying wilting (Jawaharlal *et al.*, 2012). The potential of boric acid in prolonging post-harvest life of flowers has also been reported by Mukhopadhyay *et al.*, 1980 and Karuppaiah *et al.*, 2006 in jasmine.

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# Organic Manure and Fertility Level Affects the Flowering, Yield and Quality Attributes of Okra under Heavy Clay Soil of Southern Rajasthan

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## ABSTRACT

The present experiment was conducted to find out the effects of organic manures and different dose of inorganic fertilizers on yield and quality of okra cv. Varsha Upkar. The experiment consisted of 21 treatment combinations with four organic manures (control, 15 t FYM. ha<sup>-1</sup>, 5 t vermicompost. ha<sup>-1</sup>, 5 t poultry manure. ha<sup>-1</sup>) and three fertility levels (control, 75% RDF and 100% RDF) in Factorial Randomized Block Design (FRBD) with three replications. The results showed that the application of poultry manure 5t/ha significantly found superior which took minimum days to first flower appearance (38.90), days to 50 percent flowering (44.70), maximum number of fruits per plant, fruit weight (18.28g), yield parameters (385.99 g/plant), chlorophyll content and crude protein. Further, 100% RDF and combined application (100% RDF + poultry manure 5 t/ha) were also exhibited better results of flowering, yield and biochemical parameters.

## HIGHLIGHTS

- Poultry manure is a good source of organic manure for okra plant growth and yield.
- Application of poultry manure significantly enhanced the number of flowers and fruits per plant.
- Poultry manure found suitable in enhancing the yield of okra.
- Biochemical traits of okra fruits such as chlorophyll and protein also influenced by the application of poultry manure.

**Keywords:** Okra, poultry manure, inorganic, chlorophyll, crude protein

Vegetables are the protective food and consumed worldwide due to their numerous health benefits. Okra (*Abelmoscus esculentus* L.) is an important vegetable, belongs to the family Malvaceae and grown in tropical and subtropical tracts globally as well as in India (Akanbi 2002). This is cultivated for its edible green seed pod. Okra is a good source of vitamins, calcium, potassium, and other minerals. It has been reported that okra contains good amount of potassium, sodium, calcium, iron zinc and other

elements (Adekia *et al.* 2019). Due to its high iodine content, consumption of okra is good for the control of goiter. In India, okra is cultivated over an area of 5.11 lakh ha area with production of 62.1 lakh MT (NHB, 2018-19). In Rajasthan, it is grown extensively

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in the district of Sirohi, Bundi, Jodhpur, Sikar, Jhalawar, Nagaur, Bharatpur and Jhunjhunu and covers around 5.71 thousand hectares area with annual production of 49.68 thousand metric tonnes.

Despite of popularity among stakeholders, okra produces fewer yields under soil condition of Southern Rajasthan. Being highly black cotton and heavy soil, the productivity of okra is very less. The soil of southern humid parts of Rajasthan contains high soil pH (8.0 to 9.5) and low organic matter content. Soil organic matter is a crucial factor in soil for sustainable crop production (Agboola 1990). Improper use of chemical fertilizers declined the soil fertility and leached out the nutrients from the soil. Besides, continuous use of inorganic fertilizers also changed the physical and chemical status of soil thus resulting in reducing fertility level and poor crop production. Use of organic manures, poultry manures, vermicompost and farm yard manure enriches organic matter content subsequently enhances yield and quality attributes in various vegetables (Sameera *et al.* 1995). Poultry manure is an excellent organic fertilizer, is concentrated source of nitrogen and other essential nutrients. It has direct effect on plant growth. It is well documented that it is an excellent source of fertilizer and increased nutrient uptake (Abusaleha 1992). In addition to that, organic manures also positively improve the health of soil as well as ecosystem. The use of chemical fertilizers in an integrated manner with organic manures could enhance the yield and productivity of crops with minimum disturbance of soil. In the climate changing scenario, the judicious use of chemical fertilizers with organic manures is need of the hour for sustainable crop production (Chowdhury *et al.* 2014). Among the nutrient N, P and K are the essential nutrients which are needed for growth, flowering and fruiting (Solangi *et al.* 2005). However, these studies are insufficient to validate the existing findings under humid zone of Southern Rajasthan. This study was investigated to find out the impact of various organic manures and fertility level on Okra yield and biochemical attributes under heavy clay soil.

## MATERIALS AND METHODS

### Location

The present experiment was conducted at farm of Department of Vegetable Science, College of

Horticulture & Forestry, Jhalrapatan city, Jhalawar during *kharif* season, 2019-20. The present experiment was conducted on okra crop cv. Varsha Uphar.

### Treatments Details

The treatments manures viz. FYM, Poultry manures and vermicompost were applied in the soil before sowing of seeds. The inorganic manures N:P:K in the various levels viz. control, 75% RDF and 100% RDF were applied through urea, di-ammonium phosphate (DAP) and murate of potash (MOP) respectively. The recommended dose of NPK for okra crop was 60:32:30 kg ha<sup>-1</sup>, respectively. For each fertilizer treatment combination, the NPK dose were calculated and applied timely. Full dose of phosphorus and potassium and half dose of nitrogen are applied as basal dose just before sowing and rest half dose of nitrogen was applied in two splits i.e. 30 and 45 days after sowing. The details of treatments combinations are given underneath:-

Sl. No.	Treatment Notation	Treatment Combination
1	O <sub>0</sub>	Control
2	I <sub>1</sub>	75% RDF
3	I <sub>2</sub>	100% RDF
4	O <sub>1</sub>	FYM 7.5 tons/ha
5	O <sub>2</sub>	FYM 15 tons/ha
6	O <sub>3</sub>	Vermicompost 2.5 tons/ha
7	O <sub>4</sub>	Vermicompost 5 tons/ha
8	O <sub>5</sub>	Poultry manure 2.5 tons/ha
9	O <sub>6</sub>	Poultry manure 5 tons/ha
10	I <sub>1</sub> O <sub>1</sub>	75% NPK + FYM 7.5 tons/ha
11	I <sub>1</sub> O <sub>2</sub>	75% NPK + FYM 15 tons/ha
12	I <sub>1</sub> O <sub>3</sub>	75% NPK + Vermicompost 2.5 tons/ha
13	I <sub>1</sub> O <sub>4</sub>	75% NPK + Vermicompost 5 tons/ha
14	I <sub>1</sub> O <sub>5</sub>	75% NPK + Poultry manure 2.5 tons/ha
15	I <sub>1</sub> O <sub>6</sub>	75% NPK + Poultry manure 5 tons/ha
16	I <sub>2</sub> O <sub>1</sub>	100% NPK + FYM 7.5 tons/ha
17	I <sub>2</sub> O <sub>2</sub>	100% NPK + FYM 15 tons/ha
18	I <sub>2</sub> O <sub>3</sub>	100% NPK + Vermicompost 2.5 tons/ha
19	I <sub>2</sub> O <sub>4</sub>	100% NPK + Vermicompost 5 tons/ha
20	I <sub>2</sub> O <sub>5</sub>	100% NPK + Poultry manure 2.5 tons/ha
21	I <sub>2</sub> O <sub>6</sub>	100% NPK + Poultry manure 5 tons/ha

The number of days taken for plants to first flowering in each plot and replication was recorded and expressed in days. The number of days taken for 50 per cent of flowering in each plot was



recorded and expressed in days. The number of fruit of five tagged plants was counted and the average was worked out. Fruit weight was calculated by weighing with electronic weighing machine. The estimated yield per plant was calculated by multiplying fruit weight with total number in a individual plant.

### Chlorophyll and Crude Protein Content

Chlorophyll content in leaves was measured as per method suggested by Sadasivam and Manickam (1997) and expressed in mg/100g.

The amount of protein in okra fruit was calculated in per cent. The formula used for calculating the protein is as under:

$$\text{Protein \%} = \text{Nitrogen\%} \times 6.25$$

This is based on the assumption that plant protein contains 16 per cent nitrogen. Estimation of nitrogen was done by colorimetric method as suggested by Snell and Snell (1949) using the Spectronic-20 (Model SL-177).

### Statistical Analysis

The experimental data are to be recorded during the course of investigation for various characters under study with appropriate statistical analysis (Panse & Sukhatme 1985) along with suitable interpretation.

## RESULTS AND DISCUSSION

### Days Taken to Flower Appearance

The data recorded for days to first flower appearance was influenced by application of organic and inorganic manures over control, which showed significant variation over control (Table 1). Minimum days to first flower (38.90) were recorded in treatment  $O_6$ , whereas maximum was recorded in control (44.53). Similarly, the application of inorganic manures with treatment  $I_2$  (100% RDF) took minimum days to first flower appearance with 40.70 over control with 43.29. Further, the application of organic manures with inorganic manures minimized the number of days to first flower appearance over control. Minimum days to first flower appearance was recorded in treatment  $O_6I_2$  (100% RDF + PM@ 5 ton) with 34.87 and maximum in control (47).

The days taken for 50 per cent flowering exhibited highly significant variation over control with application of organic and inorganic manures. The application of organic manures significantly affected the days taken to 50 percent flowering over control. Minimum days taken for 50 per cent flowering was recorded in  $O_6$  (44.70) and it was found statistically at par with treatment  $O_5$  (46.06),  $O_3$  (46.64),  $O_4$  (46.81), and  $O_2$  (47.04), whereas maximum days taken for 50 per cent flowering was recorded in control with 50.69. Further, the application of inorganic fertilizer exhibited significant result over control. Minimum days were taken for 50 per cent flowering was recorded in  $I_2$  (100% RDF) with 45.13 and maximum in control with 49.24. The combination of organic and inorganic fertilizers also exhibited significant variation over control. The treatment  $O_6I_2$  (100% RDF + PM@ 5 ton) showed minimum days to 50% flowering over control i.e., 39.97 and 57.00, respectively. Days to first flower appearance is also an important trait which determines its flowering and earliness. Addition of poultry manure makes reproductive booster nutrients and helps them to restore thus the early flowering and 50% flowering was observed in the treatment. Our research findings are in agreement with previous studies by (El-shakweer *et al.* 1998; Sanwal *et al.* 2007).

### Fruit Yield and Quality Parameters

The result presented reflects that fruit weight showed significant variation (Table 2). The application of organic manures increased significantly the fruit weight over control. Maximum fruit weight was recorded in  $O_6$  (18.28), and it was found statistically at par with  $O_4$  i.e., 18.02 whereas minimum fruit weight was found in control (13.79). Similarly, the application of inorganic manures also increased the fruit weight significantly over control. Maximum fruit weight was recorded in  $I_2$  (100% RDF) with 20.11 g and minimum in control with 12.44 g. The interaction effect of organic and inorganic manures also showed significant effect on fruit weight over control. Maximum fruit weight 21.61 g was recorded in treatment  $O_4I_2$  (100% RDF + VC 5 ton) over control with 9.14 g. A perusal of data regarding to fruit yield per plant presented and revealed that fruit yield per plant was significantly affected by the application of different organic



manures and fertility levels. The application of organic manures significantly increased the fruit yield per plant over control. Maximum fruit yield per plant was recorded in O<sub>6</sub> (385.99), and it was found statistically at par with O<sub>4</sub> (381.57) and O<sub>5</sub> (361.90), whereas minimum fruit yield per plant was found in control (277.06). Further, inorganic manures also played an important role in increasing the fruit yield per plant. Maximum fruit yield per plant was recorded in I<sub>2</sub> (100% RDF) i.e., 430.99g and minimum in control i.e., 243.75g. Results with respect to interactive effect of organic and inorganic showed that maximum fruit yield per plant (462.33g) was recorded in treatment O<sub>6</sub>I<sub>2</sub> (100% RDF + PM 5 ton) and it was found statistically at par with treatment O<sub>4</sub>I<sub>2</sub>, O<sub>2</sub>I<sub>2</sub>, O<sub>5</sub>I<sub>2</sub> and O<sub>3</sub>I<sub>2</sub> while minimum was recorded in control (173.34 g). The result of present study has with respect to estimated fruit yield has been presented which reflects that fruit yield q/ha showed significant variation. The increased yield and yield attributes with poultry manure might be because of rapid availability and utilization of nitrogen for various internal plant processes for carbohydrates production. Later on these carbohydrates undergo hydrolysis and get converted in to reproductive sugars which ultimately helped in increasing yield. Singh and Srivastava (1970) reported that high carbohydrates content due to application of poultry manure might be attributed to balanced C:N ratio and increased activity of plant metabolisms. These results are in accordance with the findings of Naidu *et al.* (2002) and Islam *et al.* (2012). The increased balanced C: N ratio might have increased the synthesis of carbohydrates with ultimate improvement in yield and yield attributes Chander *et al.* (2005), Kondappa *et al.* (2009), Sharma *et al.* (2009) and Yadav and Yadav (2010). The application of 100 per cent RDF resulted in the highest and significantly more values of yield and yield attributes. These findings are similar of those Garhwal *et al.* (2007), Vennila and Jayanthi (2008a), Sharma *et al.* (2009) in okra crop. The reason for enhancement in yield attributes could again be ascribed to the role which might have been played by the nutrients supplied to the plants. The quality yield depends on several management factors such as fertilizers and nutrient management, water, light, etc. but the role of fertilizers and nutrients is crucial and important in determining the fruit quality as well as yield. It is relevant to mention

here that adequate supply of nitrogen to plants not only promotes the manufacture of food but also its subsequent partitioning in skin. Similarly, phosphorus play a unique role in laying down the floral primordial. Potassium facilitates translocation of photosynthates towards various organs of plant body (Marschner 1995). The application of NPK favoured the metabolic and auxin activities in plant and ultimately resulted in increased fruit size, number of fruits per plant and yield per plant (Garhwal *et al.* 2007).

### Biochemical Parameters

A critical examination of the data pertaining to chlorophyll content in leaves has been presented in table. Chlorophyll content in leaves was significantly affected by application of different organic manures. The treatment O<sub>6</sub> (PM 5 ton) was recorded significantly higher chlorophyll content (0.82 mg g<sup>-1</sup>) in leaves and it was found statistically at par with treatment O<sub>4</sub> (0.79), whereas lower chlorophyll found in control (0.66 mg/100 g). Further, application of inorganic manures also showed significant effect over control on chlorophyll content in leaves. The treatment I<sub>2</sub> (100% RDF) resulted in significantly higher chlorophyll content (0.92 mg g<sup>-1</sup>) in leaves and lower in control I<sub>0</sub> with (0.60 mg g<sup>-1</sup>). However, the interactive effect of organic and inorganic manures also recorded significant effect on chlorophyll content in leaves over control. Maximum chlorophyll (1.05 mg g<sup>-1</sup>) was found in O<sub>6</sub>I<sub>2</sub> (100% RDF + PM 5 ton) and minimum in control O<sub>6</sub>I<sub>0</sub> (0.52 mg g<sup>-1</sup>) respectively but O<sub>4</sub>I<sub>2</sub> was found statistically at par with O<sub>6</sub>I<sub>2</sub>. Leaf chlorophyll content is major parameter which imparts greenness to the leaf as well as also acts as reservoir of pigment for pod development. Plant chlorophyll is directly correlated with nutrient assimilation and better photosynthesis process. The organic manures enhance the vegetative flushes, which favors more photosynthesis along with some important micro and macro nutrient such as iron and Mg. The interactions of these minerals is a complex phenomenon but directly indirectly involved in photosynthetic processes (Senjobi *et al.* 2010).

It is evident from data in Table that crude protein exhibited significant variation over control with application of organic and inorganic manures.

**Table 1:** Effect of organic manure and inorganic fertilizer level on days to first flower appearance of okra cv. Varsha Uphar

Treatments	O <sub>0</sub> (Control)	O <sub>1</sub> FYM 7.5 Tons/ha	O <sub>2</sub> FYM 15 Tons/ha	O <sub>3</sub> VC 2.5 Tons/ha	O <sub>4</sub> VC 5 Tons/ha	O <sub>5</sub> PM 2.5 Tons/ha	O <sub>6</sub> PM 5 Tons/ha	Mean
I <sub>0</sub> (Control)	47.00	45.60	40.67	42.97	44.47	41.87	40.47	43.29
I <sub>1</sub> (75% RDF)	44.00	42.47	44.27	41.50	41.47	41.73	41.37	42.40
I <sub>2</sub> (100% RDF)	42.60	42.83	42.10	40.83	41.37	40.33	34.87	40.70
Mean	44.53	43.63	42.34	41.77	42.43	41.31	38.90	42.13
	SE m ±					C.D. (p=0.05)		
I	0.43					1.25		
O	0.66					1.91		
I×O	1.15					3.30		

Data represents the mean value of Factor I (organic manure) and Factor-II (Inorganic RDF) and their interactions. (FYM-Farm Yard Manure; VC-Vermicompost; PM-Poultry Manure).

**Table 2:** Effect of organic manure and inorganic fertilizer level on days to 50 per cent flowering of okra cv. Varsha Uphar

Treatments	O <sub>0</sub> (Control)	O <sub>1</sub> FYM 7.5 Tons/ha	O <sub>2</sub> FYM 15 Tons/ha	O <sub>3</sub> VC 2.5 Tons/ha	O <sub>4</sub> VC 5 Tons/ha	O <sub>5</sub> PM 2.5 Tons/ha	O <sub>6</sub> PM 5 Tons/ha	Mean
I <sub>0</sub> (Control)	57.00	51.17	46.30	47.80	49.77	46.87	45.77	49.24
I <sub>1</sub> (75% RDF)	48.10	48.20	49.47	45.87	45.73	45.57	48.37	47.33
I <sub>2</sub> (100% RDF)	46.97	46.70	45.37	46.27	44.93	45.73	39.97	45.13
Mean	50.69	48.69	47.04	46.64	46.81	46.06	44.70	47.23
	SEm ±					C.D. (p=0.05)		
I	0.54					1.55		
O	0.83					2.37		
I×O	1.43					4.10		

Data represents the mean value of Factor I (organic manure) and Factor-II (Inorganic RDF) and their interactions. (FYM-Farm Yard Manure; VC-Vermicompost; PM-Poultry Manure).

**Table 3:** Effect of organic manure and inorganic fertilizer level on fruits weight (g) of okra cv. Varsha Uphar

Treatments	O <sub>0</sub> (Control)	O <sub>1</sub> FYM 7.5 Tons/ha	O <sub>2</sub> FYM 15 Tons/ha	O <sub>3</sub> VC 2.5 Tons/ha	O <sub>4</sub> VC 5 Tons/ha	O <sub>5</sub> PM 2.5 Tons/ha	O <sub>6</sub> PM 5 Tons/ha	Mean
I <sub>0</sub> (Control)	9.14	11.64	14.58	11.67	12.66	12.76	14.60	12.44
I <sub>1</sub> (75% RDF)	12.65	14.71	17.38	20.37	19.79	19.63	19.86	17.77
I <sub>2</sub> (100% RDF)	19.59	19.73	20.30	19.64	21.61	19.53	20.38	20.11
Mean	13.79	15.36	17.42	17.23	18.02	17.31	18.28	16.77
	SEm ±					C.D. (p=0.05)		
I	0.15					0.43		
O	0.23					0.66		
I×O	0.40					1.15		

Data represents the mean value of Factor I (organic manure) and Factor-II (Inorganic RDF) and their interactions. (FYM-Farm Yard Manure; VC-Vermicompost; PM-Poultry Manure).

Maximum crude protein was recorded in O<sub>6</sub> (2.21), whereas minimum crude protein was found in control (1.68). Further, maximum crude protein was recorded in 100% RDF with 2.46. Results with respect to interactive effect of organic and inorganic showed that maximum crude protein (2.74) was recorded in treatment 100% RDF + PM 5 ton and it was found statistically at par with treatment 100%

RDF + VC 5 ton, 100% RDF + FYM 15 ton, 100% RDF + PM 2.5 ton. The protein content is composed of several amino acids which are influenced with field management, nutrients, and environmental factors. The composition of protein also helps in judging the quality produce and this is one of the important index for quality trait among biochemical parameters. The soil application of organic manures

**Table 4:** Effect of organic manure and inorganic fertilizer level on fruits yield per plant (g) of okra cv. Varsha Uphar

Treatments	O <sub>0</sub> (Control)	O <sub>1</sub> FYM 7.5 Tons/ha	O <sub>2</sub> FYM 15 Tons/ha	O <sub>3</sub> VC 2.5 Tons/ha	O <sub>4</sub> VC 5 Tons/ha	O <sub>5</sub> PM 2.5 Tons/ha	O <sub>6</sub> PM 5 Tons/ha	Mean
I <sub>0</sub> (Control)	173.34	225.05	250.83	227.17	285.02	253.36	291.46	243.75
I <sub>1</sub> (75% RDF)	250.72	296.92	353.87	404.46	403.85	405.82	404.19	359.97
I <sub>2</sub> (100% RDF)	407.11	412.87	431.01	421.25	455.85	426.52	462.33	430.99
Mean	277.06	311.61	345.24	350.96	381.57	361.90	385.99	344.90
	SEm ±					C.D. (p=0.05)		
I	6.44					18.43		
O	9.85					28.15		
I×O	17.06					48.77		

Data represents the mean value of Factor I (organic manure) and Factor-II (Inorganic RDF) and their interactions. (FYM-Farm Yard Manure; VC-Vermicompost; PM-Poultry Manure).

**Table 5:** Effect of organic manure and inorganic fertilizer level on chlorophyll content in leaves (mg/100g) of okra cv. Varsha Uphar

Treatments	O <sub>0</sub> (Control)	O <sub>1</sub> FYM 7.5 Tons/ha	O <sub>2</sub> FYM 15 Tons/ha	O <sub>3</sub> VC 2.5 Tons/ha	O <sub>4</sub> VC 5 Tons/ha	O <sub>5</sub> PM 2.5 Tons/ha	O <sub>6</sub> PM 5 Tons/ha	Mean
I <sub>0</sub> (Control)	0.52	0.54	0.59	0.57	0.65	0.61	0.67	0.60
I <sub>1</sub> (75% RDF)	0.64	0.69	0.71	0.77	0.73	0.79	0.75	0.73
I <sub>2</sub> (100% RDF)	0.82	0.85	0.94	0.88	1.00	0.91	1.05	0.92
Mean	0.66	0.69	0.75	0.74	0.79	0.77	0.82	0.75
	SEm ±					C.D. (p=0.05)		
I	0.008					0.021		
O	0.01					0.03		
I×O	0.02					0.05		

Data represents the mean value of Factor I (organic manure) and Factor-II (Inorganic RDF) and their interactions. (FYM-Farm Yard Manure; VC-Vermicompost; PM-Poultry Manure).

**Table 6:** Effect of organic manure and inorganic fertilizer level on crude protein content (%) of okra cv. Varsha Uphar

Treatments	O <sub>0</sub> (Control)	O <sub>1</sub> FYM 7.5 Tons/ha	O <sub>2</sub> FYM 15 Tons/ha	O <sub>3</sub> VC 2.5 Tons/ha	O <sub>4</sub> VC 5 Tons/ha	O <sub>5</sub> PM 2.5 Tons/ha	O <sub>6</sub> PM 5 Tons/ha	Mean
I <sub>0</sub> (Control)	1.33	1.34	1.36	1.45	1.54	1.63	1.65	1.47
I <sub>1</sub> (75% RDF)	1.71	1.75	1.78	1.82	1.87	1.93	2.25	1.87
I <sub>2</sub> (100% RDF)	1.99	2.12	2.71	2.34	2.72	2.61	2.74	2.46
Mean	1.68	1.74	1.95	1.87	2.05	2.06	2.21	1.94
	SEm ±					C.D. (p=0.05)		
I	0.01					0.05		
O	0.02					0.08		
I×O	0.05					0.14		

enhances root microbial activity and nutrient assimilation along with cation exchange capacity. The mineralogy of soil alters the plant physiology and its catabolic and anabolic reactions. Certain enzymes helps I synthesis of amino acids due to higher assimilate and a strong source sink relationship. The highest value of crude protein in okra pod in our study, might be due to positive

impact of poultry manure in soil as well as in plants which favours the more synthesis of amino acids thus increased protein content. Soremi *et al.* (2017) reported that poultry manure change the soil fertility status that resulted in whole biochemical composition of soil as well plants. This also releases organic acids and increase organic content into the soil which retain the loss of various nutrients.



Our results are in agreement of previous studies by Trupiano *et al.* (2017) and Imasuen and Aisien (2015). The significant influence of NPK fertilization on N, P, K and protein content in fruits appeared to be due to improved nutrient both in the root zone and the plants system because nutrient in the plant directly related to its availability in the feeding zone and the growth of the plant. The protein content in fruits is infact a manifestation of nitrogen content. The increase in nitrogen content in fruits resulted in higher protein content in fruits. These results are in close conformity with findings of Yadav (2001), Maheswari and Haripriya (2007) and Premshekhar and Rajashree (2009). The higher content in fruits seems to be higher functional activity of roots for longer duration under the treatment. The increase in N, P and K content in fruit due to adequate fertilization have also been observed by Nanthakumar and Veeragavathatham (2003) and Selvi *et al.* (2004). The accumulation of higher protein content in the fruits might be correlated with the increased activity of nitrate reductase which helped in synthesis of certain amino acids and proteins. These results are also corroborated by the findings of Yadav *et al.* (2006) and Garhwal *et al.* (2007) in okra crop.

## CONCLUSION

In conclusion it was observed that the treatment O<sub>6</sub> 5 t poultry manure/ha most suited to okra crop cv. Varsha Uphar which enhanced the flowering parameters and yield. The combination of poultry manure and 100% RDF and solely 100% RDF found best for overall yield and quality under hard clay soil. However, further investigation need to elucidate the mechanism and mineralogy under such soil conditions.

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# Bioactive Compounds of Turmeric Powder Affected by Grinding Method and Feed Temperature

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## ABSTRACT

The turmeric used as a ground powder. Turmeric grinding is carried out in grinding mill. During grinding operation the temperature inside the grinding mill increases. The bioactive compounds along with their biological activity and stability depends on the grinding temperature. High temperature during grinding reduced the bioactive compounds along with their biological activity and stability. Four grinding methods and two feed temperature were chosen on the hypothesised mechanisms of reduction in temperature during the grinding. The effects of grinding temperature and feed temperature on the phenolic content, flavonoid content, antioxidant activity and curcumin content of turmeric ground powder were studied. Antioxidant activity, flavonoid content, curcumin content and phenolic content increased with decrease in grinding temperature. The temperature inside the grinding chamber at the end of grinding of 3 kg sample of turmeric reached to 43 °C for traditional grinding, and this was reduced to 18.33 °C for coolant circulation with low temperature feed. This reduction in grinding temperature resulted in the highest phenol content (3.13%), flavonoid content (1.43%), and antioxidant activity (59.31%) for coolant circulation with low feed temperature. Chilled water circulation with low temperature feed resulted the highest curcumin content (2.48%). This bioactive compound were significantly differed with grinding method as well as feed temperature.

## HIGHLIGHTS

- The article focuses on the effect of both moisture content and variety on the physical properties of psyllium seeds
- The moisture content and variety both had a significant effect on the physical properties of psyllium seeds
- The size, thousand seed weight, angle of repose, coefficient of static friction and terminal velocity were increased while bulk density, true density and porosity were decreased as the moisture content was increased

**Keywords:** Turmeric powder, low temperature grinding, curcumin, flavonoid, phenolic, antioxidant activity

The origin of genus *Curcuma* from the Zingiberaceae family lies in the Indo-Malayan Region spreading in the tropics of Asia to Africa and Australia (Pusglove 1968). Turmeric powder, generally used as a spice, food preservative, colouring agent, is obtained by grinding turmeric rhizome of *Curcuma longa* L. Turmeric compounds possess anti-inflammatory, anti-HIV, antibacterial, antioxidant, anti-diabetes,

and anti-carcinogenic activities (Park Chang Yang, Kyo-Yeon Lee, Khalid Gul, M. Shafiur Rahman,

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Ah-Na Kim, Jiyeon Chun, Hyun-Jin Kim & Sung-Gil Choi 2019). The important active compounds of turmeric are curcumin which may be in the form of dihydrocurcumin, demethoxycurcumin, and bisdemethoxycurcumin which are important for most of the turmeric's beneficial effects (Prathapan, Lukhman, Arumughan, Sundaresan, & Raghu 2009). Curcumin has property of anti-inflammatory activity. Curcumin is an effective inhibitor for reactive oxygen-generating enzymes like lipooxygenase/cyclooxygenase, xanthine dehydrogenase/oxidase, and inducible nitric oxide synthase (Lin, Pan & Lin-Shiau 2000). Metabolism activity of Curcumin reduces it through an NADPH dependent curcumin reductase consequential in a dihydrocurcumin. Dihydrocurcumin again reduces through an NADPH-dependent dihydrocurcumin reductase bring about in a tetrahydrocurcumin (THC) (Aggarwal, Deb, and Prasad, 2015, Khopde, Priyadarsini, Guha, Satav, Venkatesan, P., & Rao 2000).

Ancillary metabolites of plant metabolism, phenolic compounds can chelate metallic ions, scavenge free radicals during oxidative stress, and increase antimicrobial activities (Pereira *et al.* 2009). Bioactive compounds frequently store in the plant in lesser quantities and sometimes in specific cells (Finley 2005). In the middle of them certainly are recognized as phenolic, flavonoids, and essential oils, which have a wide range of biological activities such as antioxidant, anti-inflammatory, anti-aging, anti-bacterial, anti-tumor, and other functions (Karimi, Jaafar, & Ahmad, 2013; Oskoueian, Abdullah, Hendra & Karimi, 2011).

Consumer demand for healthier products having less synthetic additives is motivating for research to discover substitute sources of natural antioxidants. Natural antioxidants like phenolic and flavonoid compounds are available in plants and they are appealing a great deal of responsiveness due to amassed confirmation suggesting that they may inhibit chronic conditions, such as cancer, atherosclerosis, and neurological diseases (Marinova, D., Ribarova, F. & Atanassova, M. 2005). In the traditional grinding of spices, frictional heat is generated in the grinder. The grinding process increases the product temperature to a high level in the range of 42-95°C (Pruthi and Mishra

1963), depending on the oil and moisture content, consequently, it loses a substantial portion of its volatile oil. The fat in spices poses extra problems and is an important consideration in grinding.

By circulating water or air around the mill, temperature reduction can possible, but it is not that effective. Though cryogenic grinding is very costly. Low temperature grinding techniques can also be easily available for small mills than cryogenic grinding. It was also found that the colour and other properties of the low temperature grind material were not changed, and the flavour and nutrients of the medicines were not lost. From the statements above, there seems enough justification for low temperature grinding of turmeric to obtain a high quality product. Theoretically, though low temperature grinding of turmeric is better than ambient grinding, the available literature is scanty to support the above justification. Hence, in the present study, efforts were made to develop low temperature grinding technique for turmeric.

## MATERIALS AND METHODS

### Grinding methods

Dried turmeric rhizome of Salem variety was purchased from local market. Turmeric powder was prepared by different grinding methods viz.  $L_0$  = Ambient grinding,  $L_1$  = Grinding with ambient temperature water circulation through jacket,  $L_2$  = Grinding with chilled water circulation and  $L_3$  = Grinding with coolant circulation. The temperature inside the grinding chamber at the end of grinding were noted. The powder were collected. Ground powder of turmeric rhizome obtained by various treatments through low temperature grinding mill was packed carefully, stored to room temperature at dark, dry and hygienic place and opened at the time of analysis only. Self-sealing zip lock transparent plastic bags were used for packing after cooling the powder to room temperature. Care was taken not to allow air inside. Additional wrapping of ordinary transparent plastic cover was also followed.

Biochemical parameters of turmeric powder obtained through different treatments were determined by using standard protocols. All the samples with triplicates were analysed at a time for determination of a particular parameter.

### Preparation of low temperature feed

Pre-weighted turmeric rhizome were packed in self-sealing zip lock transparent plastic bags. Additionally, packing was wrapped by an ordinary transparent plastic cover. Packed rhizomes were kept in freezer at  $-10^{\circ} \pm 2^{\circ}$  overnight, the day before grinding. Besides that, feed was withdrawn from the freezer at the time of grinding only and once withdrawn, placed at feed hopper immediately. Each packet of the rhizome involved half the amount of decided sample size, which allowed one half of the sample to be in the freezer instead of the feed hopper while grinding. Care was taken to feed the other half well before completion of the first half size of sample in the feed hopper.

### Total phenol content

The phenol content of the turmeric powder was estimated as per the method given by Malick and Singh (1980). 0.1 g of turmeric powder (weighed to the nearest 0.0001 g) was extracted in 10 ml of 80% ethanol. Centrifugation was done and, the supernatant was collected. Then 0.1 ml of aliquot was pipetted out into a test tube and was evaporated to dryness. After drying, the residue was dissolved in 1 ml of distilled water. Then 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard into a series of test tubes was pipetted out and the total volume of each was made up to 1 ml using distilled water. 1 ml of distilled water in a test tube was set for a blank solution. 0.5 ml of Folin-Ciocalteu reagent was then added to each tube including blank. After three minutes, 2 ml of 20%  $\text{Na}_2\text{CO}_3$  solution was added to each. The solution was mixed thoroughly and was placed in a boiling water bath for exactly one minute. After cooling to room temperature, colour was read at 650 nm using a UV-Visible spectrophotometer (Thermo Scientific, GENESYS 50). At last, the percentage of total phenol was calculated by preparing a standard curve of catechol. The following formula was used.

Total phenol (%) =

$$\frac{\text{GF} \times \text{OD} \times \text{Total volume (ml)} \times 10^{-6}}{\text{Sample aliquot (ml)} \times \text{Sample Weight (g)}} \quad \dots(1)$$

Where, GF = Graph factor

OD = Optical density

### Total flavonoid content

Aluminium chloride colorimetric method was used to determine total flavonoid content of turmeric powder, as given by Chang *et al.* (2002). Quercetin was used for preparation of calibration curve. Different diluted quercetin standard solutions (10, 25, 50 and 100  $\mu\text{g/ml}$ ) were prepared in 80% ethanol. The sample was also extracted in ethanol. From each tube, 0.5 ml aliquot was pipetted out in separate test tubes. Then 1.5 ml of 95% ethanol followed by 0.1 ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate was added to each tube. Total volume in each tube was made up to 5 ml by adding 2.8 ml distilled water. For preparation of blank solution, aluminium chloride solution was substituted by the same amount of distilled water. After incubation at room temperature for 30 minutes, absorbance was measured at 415 nm by UV-Visible spectrophotometer (Thermo Scientific, GENESYS 50). The quantity of flavonoid was then expressed in mg equivalent of quercetin by using the following equation.

$$\text{Total flavonoid (\%)} = \frac{C \times V}{W} \times 100 \quad \dots(2)$$

Where, C = Concentration of sample extrapolated from calibration curve (mg/ml)

V = Volume of sample extract (ml)

W = Weight of sample extracted (mg)

### Antioxidant activity

The aluminium chloride colorimetric method was used to determine the total flavonoid content of turmeric powder, as given by Chang *et al.* (2002). Quercetin was used for the preparation of the calibration curve. Different diluted quercetin standard solutions (10, 25, 50, and 100  $\mu\text{g/ml}$ ) were prepared in 80% ethanol. The sample was also extracted in ethanol. From each tube, 0.5 ml aliquot was pipetted out in separate test tubes. Then 1.5 ml of 95% ethanol followed by 0.1 ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate was added to each tube. The total volume in each tube was made up to 5 ml by adding 2.8 ml distilled water. For the preparation of the blank solution, aluminium chloride solution was substituted with the same amount of distilled water. After incubation at room temperature for 30

minutes, absorbance was measured at 415 nm by a UV-Visible spectrophotometer (Thermo Scientific, GENESYS 50). The quantity of flavonoid was then expressed in mg equivalent of quercetin by using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100 \quad \dots(3)$$

### Curcumin content

The curcumin content of the turmeric powder was estimated as per the method given by Geethanjali *et al.* (2016). 1 g of the turmeric powder was accurately weighed and transferred into 500 ml round bottle flask. 75 ml acetone was added up to the mark. It was refluxed for 1.5 hour, after which it was filtered and made up to 200 ml with acetone. From this further 1ml was taken and made up to 100ml adding acetone in a standard flask. The UV spectral reading for this solution was recorded under 420 nm using UV-Visible spectrophotometer (Thermo Scientific, GENESYS 50). A UV spectrum for standard curcumin was recorded. The obtained absorption value of turmeric powder samples was compared with the standard value of curcumin. The percentage curcumin in samples was calculated using the formula:

$$\text{Curcumin (\%)} = \frac{D_s \times A_s}{100 \times W_s \times 1650} \times 100 \quad \dots(4)$$

where,  $D_s$  - sample dilution volume (i.e.,  $200 \times 100 = 20000$  ml);  $W_s$  - sample weight (g);  $A_s$  - absorbance of the sample; 1650 - standard value calculated by experts.

### STATISTICAL ANALYSIS

The observed data were subjected to analysis of variance (ANOVA) using Factorial Completely Randomized Design at 5% level of significance ( $p < 0.05$ ).

### RESULTS AND DISCUSSION

#### Temperature inside the grinding chamber at the end of grinding

Turmeric is used as ground powder. During grinding operation, heat will be generated and hence

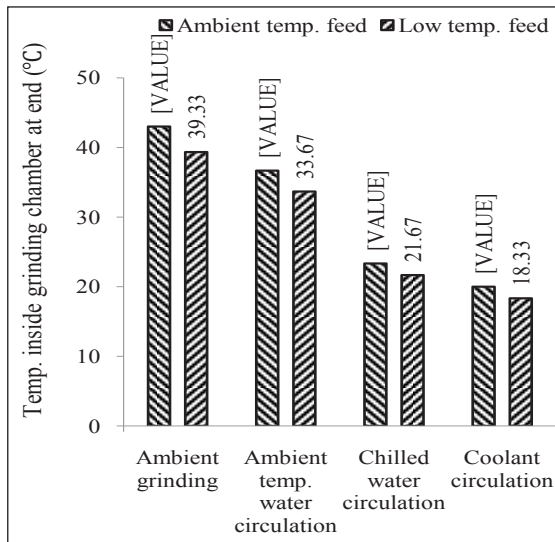
temperature inside the grinding chamber will rise. It is necessary to reduce the grinding temperature. In this different treatment, grinding method affects significantly on the value of temperature inside the grinding chamber at the end of grinding. The highest temperature ( $41.17^\circ$ ) was found for the grinding treatment  $L_0$ . Significantly, the lowest temperature ( $19.17^\circ$ ) was found for the grinding treatment  $L_3$ . The effect of feed temperature ( $p < 0.05$ ) on the same parameter was also found significant (Table 1). The significant highest value found was  $35.75^\circ$  for ambient temperature feed ( $T_0$ ). The value in case of low temperature feed ( $T_1$ ) was significantly lowest, i.e.  $34.08^\circ$ . In addition to that, the interaction effect of grinding method and feed temperature ( $L \times T$ ) on the value of temperature inside the grinding chamber at the end of grinding was found non-significant.

**Table 1:** Effect of grinding method temperature inside grinding chamber at the end of grinding

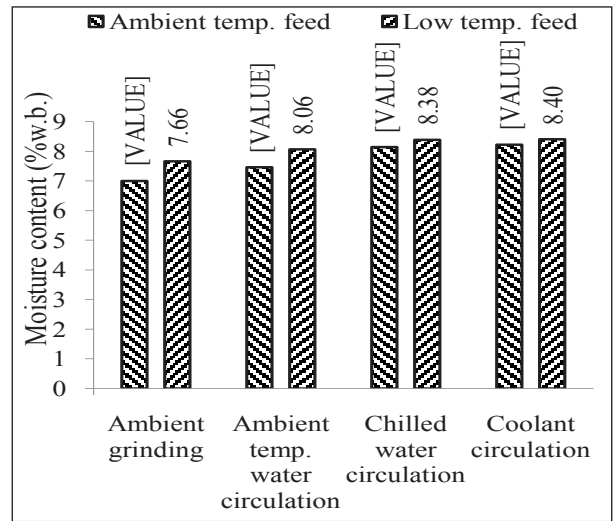
Effect	Temperature inside grinding chamber at the end ( $^\circ$ )
<b>Grinding method (L)</b>	
Ambient temperature grinding ( $L_0$ )	41.17
Ambient temperature water circulation ( $L_1$ )	35.17
Chilled water circulation ( $L_2$ )	22.50
Coolant circulation ( $L_3$ )	19.17
S. Em $\pm$	0.4787
C. D. at 5%	1.4352
<b>Feed temperature (T)</b>	
Ambient temperature feed ( $T_0$ )	35.75
Low temp. feed ( $T_1$ )	34.08
S. Em $\pm$	0.3333
C. D. at 5%	0.9994
<b>Interaction (L*T)</b>	
S. Em $\pm$	0.6667
C. D. at 5%	NS
C. V%	3.307

Number of replications,  $n=3$

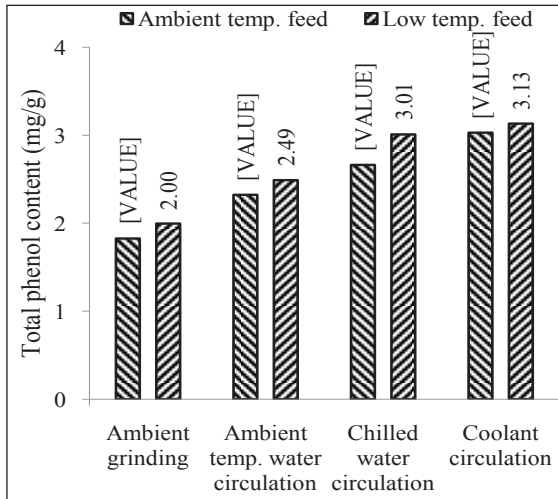
The mean values ( $n=3$ ) for this parameter for all the treatments are graphically presented in the following figure (Fig. 1(a)). It varied from  $43.00^\circ$  for treatment combination  $L_0T_0$  to  $18.33^\circ$  for treatment combination  $L_3T_0$ . This shows the effect of circulation of liquid around the grinding chamber and temperature of feed material.



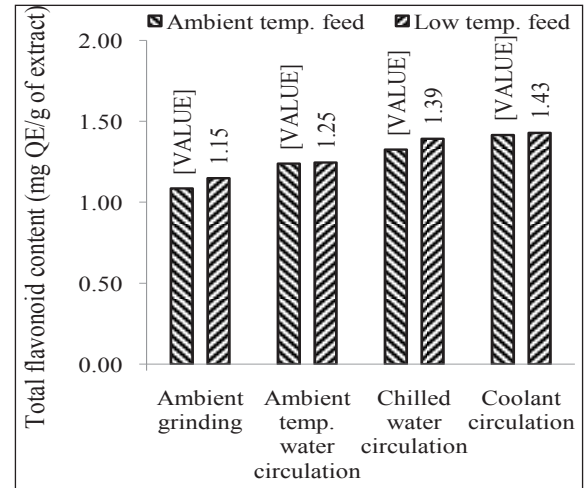
(a) temperature inside the grinding chamber



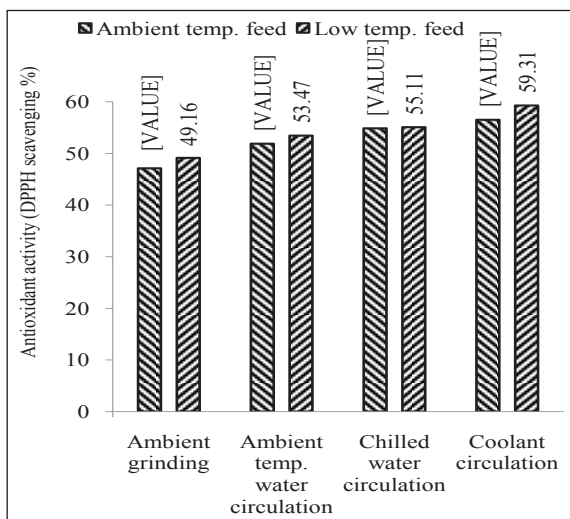
(b) moisture content



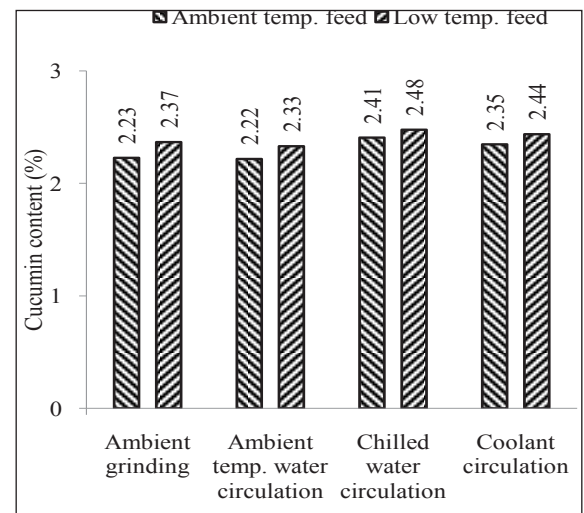
(c) total phenol content



(d) total flavonoid content



(e) Antioxidant activity



(f) Curcumin content

**Fig. 1:** Effect of various treatments on (a) temperature inside the grinding chamber and (b) Moisture content, (c) Total phenol content, (d) Total flavonoid content, (e) Antioxidant activity (f) Curcumin content in turmeric powder

Number of replications,  $n = 3$



The fall in temperature in grinding chamber becomes substantial with the change in grinding method compared to the change in feed temperature keeping the grinding method same, especially when jumping to chilled water and coolant circulation methods from ambient temperature water circulation. Possibly, resting of considerable amount of time in the feed hopper increased the temperature of low temperature feed, which in turn diminished its effect to some extent. This may be due to continuous absorption of heat generated during grinding operation. Additionally, lowering the temperature of liquid and circulating around the grinding chamber results in appreciable falling of final temperature inside the grinding chamber. That was caused by absorption of more amount of heat generated during grinding operation due to increase in the value of difference in temperature between grinding chamber and circulating liquid around.

### Moisture content

The grinding method affects significantly the value of moisture content of ground product at a 5% level of significance. The lowest value (7.33%) was found for the grinding treatment  $L_0$  while the grinding treatment  $L_3$  exhibited higher percentages of moisture (8.31%). The effect of feed temperature on the value of moisture content was also found significant ( $p < 0.05$ ). The values found were 7.70% and 8.13% for ambient temperature and low temperature feed, respectively. In addition to individual effects, the interaction effect of the grinding method and feed temperature ( $L^*T$ ) on the same parameter was found non-significant.

The mean values ( $n=3$ ) of moisture content of ground powder for all the treatments are graphically presented in the following figure (Fig. 1(b)). The figure reveals that the value of moisture content of ground powder increases when moving from left to right, i.e. treatments involving ambient grinding to ambient, chilled water, and coolant circulation treatments. Values varied from a minimum of 7.00% for ambient grinding with ambient water circulation and low temperature feed ( $L_1T_1$ ) to a maximum of 8.40% for the treatment combination  $L_3T_1$ .

An increase in moisture content of ground powder might be attributed to the condensation of moisture with low temperature inside the grinding chamber. Further, lower temperature of ground powder might

decrease the loss of moisture in surrounding by evaporation. However, a decrease in the value of moisture content was observed in ground powder compared to the moisture content of turmeric rhizome (8.59%) for all the treatments. That might be due to the loss of moisture at higher temperatures generated during the grinding operation.

### Total phenol content

A significant difference ( $p < 0.05$ ) was observed in the phenol content of turmeric powder for different grinding methods (Table 2). The lowest value (1.91 mg/g) was found for the treatment  $L_0$  while the treatment  $L_3$  was found to have the highest value of total phenol (3.08%) in powder. Additionally, the effect of feed temperature on the value of total phenol was also found significant ( $p < 0.05$ ). The value found in the case of low temperature feed (2.66 mg/g) was higher than that of ambient temperature feed (2.46 mg/g). Instead of that, the interaction effect of grinding method and feed temperature ( $L^*T$ ) on the value of total phenol content of ground powder was found non-significant at the same level of significance.

Graphically presentation of the mean values ( $n=3$ ) of total phenol of ground powder for all the treatments is given in Fig. 1(c). The figure shows that the value of total phenol of ground powder increases when moving from treatments involving no circulation to coolant circulation treatments. Values ranged from a minimum of 1.83% for the control treatment ( $L_0T_0$ ) to a maximum of 3.13% in treatment combination ( $L_3T_1$ ).

Turmeric having a higher content of phenolic compounds has been intensively studied (Braga, Leal, Carvalho, & Meireles, 2003; Prathapan, Lukhman, Arumughan, Sundaresan, & Raghu, 2009). During grinding, different temperatures create different thermal gradients that induce varying stresses, resulting in the liberation of phenolic. Therefore, different phenolic compounds are available at different temperatures. An increase in total phenol of ground powder with moving from left to right in the graph might be due to the fall in the value of temperature inside the grinding chamber at the end of the grinding operation. As higher temperature causes degradation of phenolic compounds, it decreases total phenol content in ground powder.

**Table 2:** Effect of grinding method and feed temperature on bioactive compounds of turmeric powder

Effect	M. C. (%w.b.)	Phenol content (mg/g)	Flavonoids content (mgQE/g)	Anti-Oxidant Activity (%)	Curcumin content (%)
<b>Grinding method (L)</b>					
Without liquid circulation ( $L_0$ )	7.33	1.91	1.12	48.15	2.30
Ambient temperature water circulation ( $L_1$ )	7.76	2.41	1.24	52.69	2.27
Chilled water circulation ( $L_2$ )	8.26	2.84	1.36	55.00	2.44
Coolant circulation ( $L_3$ )	8.31	3.08	1.42	57.92	2.39
S. Em±	0.0766	0.0685	0.0167	0.7269	0.0435
C. D. at 5%	0.2296	0.2053	0.05	2.1792	0.1304
<b>Feed temperature (T)</b>					
Ambient temperature feed ( $T_0$ )	7.70	2.46	1.27	52.62	2.30
Low temp. feed ( $T_1$ )	8.13	2.66	1.31	54.62	2.40
S. Em±	0.0541	0.0484	0.0118	0.5140	0.0307
C. D. at 5%	0.1623	0.1452	0.0354	1.5409	0.0922
<b>Interaction (L*T)</b>					
S. Em±	0.1083	0.0969	0.0236	1.0279	0.0615
C. D. at 5%	NS	NS	NS	NS	NS
C. V%	<b>2.3694</b>	<b>6.5555</b>	<b>3.1779</b>	<b>3.3317</b>	<b>4.5302</b>

Number of replications,  $n=3$ .

### Total flavonoid content

Grinding method affects significantly ( $p<0.05$ ) on the value of total flavonoid content of ground product (Table 2). The lowest value (1.12 mg QE/g extract) was found for the grinding method without liquid circulation ( $L_0$ ) while the highest (1.42 mg QE/g extract) for the method involving coolant circulation around the grinding chamber ( $L_3$ ). The effect of feed temperature on the value of total flavonoid was also found statistically significant ( $p<0.05$ ). The values found in case of ambient feed and low temperature feed were 1.27 and 1.31 mg QE/g extract, respectively. Besides that, the interaction effect of grinding method and feed temperature ( $L*T$ ) on total flavonoid content of ground powder was found non-significant at the same level of significance.

The mean values of total flavonoid of ground powder for all the treatments are graphically shown in the following figure (Fig. 1(d)). Figure indicates that the value of total flavonoid of ground powder increases when moving from treatments involving ambient grinding to ambient water, chilled water and coolant circulation treatments. Values ranged from minimum of 1.09 mg QE/g extract for ambient grinding with ambient temperature feed treatment

( $L_0T_0$ ) to maximum of 1.43 mg QE/g extract in coolant circulation with low temperature feed ( $L_3T_1$ ). Increase in total flavonoid of ground powder with moving from ambient grinding with ambient temperature feed to coolant circulation grinding with low temperature feed might be due to the decrease in the elevation of temperature inside the grinding chamber at the end of grinding operation. As flavonoids are the largest group of phenolic compounds (naturally occurring) (Sulaiman and Balachandran, 2012), higher temperature engenders degradation of flavonoids and it decreases total flavonoid content in ground powder.

### Antioxidant activity

Table 2 shows that grinding method affects significantly on the value of antioxidant activity of ground ( $p<0.05$ ). The lowest value (48.15 %) was found for the treatment  $L_0$  while the highest (57.92 %) for the treatment  $L_3$ . The effect of feed temperature on the value of the same parameter was also found statistically significant ( $p<0.05$ ). The value found in case of low temperature feed (54.26%) was significantly higher with that of ambient temperature feed (52.62%). Conversely, the interaction effect of grinding method and feed



temperature ( $L^*T$ ) on the value of antioxidant activity was found non-significant.

The mean values of antioxidant activity of ground powder for all the treatments are graphically demonstrated in the Fig. 1(e). It shows that the value of antioxidant activity of ground powder increases when moving from treatments involving no circulation to ambient, chilled water and coolant circulation treatments. Values ranged from minimum of 47.14 % DPPH scavenging for control treatment ( $L_0T_0$ ) to maximum of 59.31 % DPPH scavenging in treatment combination  $L_3T_1$ .

Lowering of grinding temperature increases the antioxidant power and hence antioxidant activity in relation to the concentration of the secondary metabolites (Tuba & Gulcin, 2008). The same reason of increase in grinding chamber temperature can be concluded for decrease in DPPH scavenging per cent when moving from right to left in the graph. As phenolics are the largest group of phytochemicals which account for most of the antioxidant activity in plants (Sulaiman and Balachandran, 2012), degradation of phenolic compounds at higher temperature also caused decrease in antioxidant activity percentages in ground turmeric powder. The pattern of increase in antioxidant of ground powder is the same as total flavonoid content of ground powder. Tanvir *et al.* (2017) reported that presence of antioxidant flavonoids inhibit or interrupt the substrates' oxidation even at low concentrations, and hence it prevents oxidation by the pro-oxidants. Phenolics and flavonoids are non-enzymatic antioxidants that inactivate pro-oxidants due to reaction. Oxidative stress plays a major role in the pathogenesis of various diseases such as haemorrhage and shock, myocardial ischaemia, neuronal cell injury, hypoxia and cancer.

### Curcumin content

Curcumins are sensitive to high processing temperatures and are thus degraded during intense and/or prolonged thermal treatment (Prathapan *et al.* 2009). It was observed that grinding method affects non-significantly ( $p < 0.05$ ) on the value of curcumin content of ground product. The lowest value (2.23%) was found for the treatment ( $L_0$ ) while the highest (2.24 %) for the method involving treatment  $L_2$ . As the anti-oxidant activity, phenol content and

flavonoid content are significantly, curcumin may be available in the form of dihydrocurcumin or tetrahydrocurcumin, hence its content have not been observed significant effect.

The mean values ( $n=3$ ) of curcumin content of ground powder for all the treatments are graphically demonstrated in the Fig. 1(f). It shows that the values ranged from minimum of 2.22 % for treatment  $L_1T_0$  to maximum of 2.48 % for treatment combination  $L_3T_1$ .

It has been reported that curcumin in turmeric exhibit antioxidant activity by preventing lipid peroxidation in various cells including erythrocytes, liposomes and macrophages. In addition, the presence of phenolic groups in the structure of curcumin explains its ability to react with reactive oxygen species and reactive nitrogen species (Trujillo *et al.* 2013).

### CONCLUSION

In summary, the results of this study demonstrated that grinding temperature affects the moisture content, antioxidant activity, phenol content, and curcumin content in turmeric powder. The ideal grinding method is grinding with coolant circulation resulting in significantly the highest moisture content, phenol content, flavonoid content, and antioxidant activity which was superior to ambient grinding for the better bioactive compounds in turmeric powder.

The ambient grinding of turmeric at high temperatures led to the significant reduction of antioxidant activity, phenol content, and flavonoid content. These changes were abridged through with circulating the coolant in the jacket of the grinding chamber and hence by reducing the grinding temperature at the end of grinding. The findings of this study will be helpful for the reduction of the grinding temperature without the cryogenic principle. The results will also help retain curcumin, phenolic, flavonoids, and antioxidant activity during turmeric grinding.

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# Physical and Functional Properties of Extruded Snack Products Prepared by Blending of Defatted Peanut Flour with Corn Flour

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## ABSTRACT

Extruded snack products were prepared by blending of corn flour and defatted peanut flour using twin screw extruder. The flours were mixed and added with water put for conditioning prior to the extrusion cooking. The combined effects of feed moisture content, defatted peanut flour content, die head temperature and screw speed on the important physical (expansion ratio) and functional (water absorption index, water holding capacity and water solubility index) properties of extrudates were studied. The Response Surface Methodology (RSM) was used in designing the experiment. Since, the defatted peanut flour is poor in starch content, the flour content restricted the gelatinization and limited the expansion of the product. Defatted peanut flour was found to be suitable for the preparation of extruded snacks with the appropriate blending corn flour as a base material. The optimum treatment condition was found as 13% feed moisture content, 26% defatted peanut flour, 135 °C die head temperature and 250 rpm screw speed for the production of extruded product by blending of defatted peanut flour with corn flour.

## HIGHLIGHTS

- ① Utilization of defatted peanut flour in extrusion cooking.
- ① Physical and Functional properties of extrudates.
- ① Optimization of extrusion cooking conditions.

**Keywords:** Extruded product, Defatted peanut flour, Extrusion cooking, Functional properties

Extrusion cooking has been used increasingly in the production of food and food ingredients such as breakfast cereals, baby foods, flat breads, snacks, meat and cheese analogues and modified starches, *etc.* (Ding *et al.* 2004). Extrusion has gained popularity due to its versatility, cost of processing and high production rate. A very wide variety of products are possible by changing the ingredients, the operating conditions of the extruder and the shape of the dies. Extrusion has lower processing costs and higher productivity than other cooking or forming processes (Fellows, 2000). Due to lower moisture content, the shelf life of extruded products are enhanced significantly. Further, this products

are nutritionally rich and are microbiologically safe (Pathak and Kochhar 2018).

The combination of high temperature and high shear in extrusion cooking can be used as an effective cooking method to transform raw ingredients into ready-to-eat snack products. The acceptability and nutritional quality of the final product is not only determined by feed ingredient, but also manipulation of extrusion parameters including

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feed moisture, screw speed, screw configuration, and temperature. Feed moisture has been found to be the main parameter in governing the texture of extrudates because it substantially influences the rheological properties of the molten starch. Moisture acts as a plasticizer, which reduces the viscosity and mechanical energy dissipation during extrusion. The effect of screw speed on the quality of extrudate is complex and highly dependent on temperature. A higher screw speed results in greater mechanical energy, or frictional heat, which leads to an increase in product temperature. The combination of shear and temperature can lead to a change in rheological properties of the melt and therefore affect the texture of extrudate (Choton *et al.* 2020).

Successful production of ready-to-eat snack products requires close control of many extrusion parameters including feed moisture, feed rate, barrel temperature, screw speed and barrel temperature. These process variables determine the transformation of raw materials during processing, which then influence the rheological properties of plasticizer melt in the extruder. Understanding the relationships between the ingredients is quite necessary to achieve desired product quality targets and to develop new products (Forsido and Ramaswamy 2011).

At present human are becoming more and more health conscious thus incorporating of ingredients in snack products becoming necessary. Hence, the focus of this research was to enhance the nutritional and functional properties of extrudates for the development of snack product. To achieve that, specific blends of defatted peanut flour and corn flour subjected to twin-screw extrusion process under identical set of processing parameters with the objective of testing the feasibility of defatted peanut flour in the production of extruded product.

## MATERIALS AND METHODS

### Raw materials

The corn grains required for the research work was obtained from the local market of Junagadh city. The corn grains were cleaned manually to remove all impurities and then coarse grinded using stone mill. The flour was then sieved to obtain uniform particle size flour. The flour was then packed in polyethylene bag and stored in refrigerator. The

defatted peanut flour was purchased from Nutrinity Foundation, Junagadh. It was available in the vacuum packed bag in the fine powder form.

### Proximate composition of raw materials

The biochemical characteristics viz. moisture content, carbohydrate, protein, fat and ash content, of the corn flour and defatted peanut flour were determined as per the standard procedures. Moisture content was determined by oven drying method according to AOAC (2005). Carbohydrate content of the prepared flour was determined by Phenol Sulphuric acid method for total carbohydrate (Nielsen 2010). Protein content of raw flour as well as extruded product was determined by Microkjeldahl method AOAC (1965). Fat content of the composite flour in the sample was determined by Soxhlet extraction method described by AOAC (1965). All analysis are expressed as the mean ( $\pm$ SD) of triplicate analysis. Ash content of the flour was determined using muffle furnace as described by AOAC (2005).

### Experimental design

The Response Surface Methodology (RSM) is an empirical statistical modelling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariable equations simultaneously. It was used for designing of the experiment (Myers, 1976; Khuri and Cornell, 1987; Montgomery, 2001). A four-factor five-level Central Composite Rotatable Design (CCRD) with quadratic model was employed to study the combined effect independent variables on different response variables. The levels as selected for the independent parameters along with their coded and actual values are presented in Table 1 and the treatment combination are given in the Table 2.

The second order polynomial coefficients were calculated by using the software package Design Expert version 10 (STAT-EASE Inc., Minneapolis, MN, USA) to estimate the responses of the dependent variable.

### Extruded product preparation

Extrusion trials were performed using a co-rotating twin-screw extruder (Basic Technology Pvt. Ltd.,

**Table 1:** Independent parameters and their coded and actual values employed for the preparation of extruded product

Sl. No.	Parameters	Code	Coded levels				
			-2	-1	0	+1	+2
1	Feed moisture content (% w.b.)	(X <sub>1</sub> )	10	13	16	19	22
2	Peanut flour (%)	(X <sub>2</sub> )	10	20	30	40	50
3	Die head temperature (°C)	(X <sub>3</sub> )	90	105	120	135	150
4	Screw speed (rpm)	(X <sub>4</sub> )	100	150	200	250	300

**Table 2:** Matrix of experimental central composite rotatable design for preparation of extruded products

Treatment No.	Coded variables				Uncoded variables			
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	FMC (X <sub>1</sub> ) % (w.b.)	DPF (X <sub>2</sub> ) (%)	DHT (X <sub>3</sub> ) (°C)	Screw speed (X <sub>4</sub> ) (rpm)
T <sub>1</sub>	-1	-1	-1	-1	13	20	105	150
T <sub>2</sub>	1	-1	-1	-1	19	20	105	150
T <sub>3</sub>	-1	1	-1	-1	13	40	105	150
T <sub>4</sub>	1	1	-1	-1	19	40	105	150
T <sub>5</sub>	-1	-1	1	-1	13	20	135	150
T <sub>6</sub>	1	-1	1	-1	19	20	135	150
T <sub>7</sub>	-1	1	1	-1	13	40	135	150
T <sub>8</sub>	1	1	1	-1	19	40	135	150
T <sub>9</sub>	-1	-1	-1	1	13	20	105	250
T <sub>10</sub>	1	-1	-1	1	19	20	105	250
T <sub>11</sub>	-1	1	-1	1	13	40	105	250
T <sub>12</sub>	1	1	-1	1	19	40	105	250
T <sub>13</sub>	-1	-1	1	1	13	20	135	250
T <sub>14</sub>	1	-1	1	1	19	20	135	250
T <sub>15</sub>	-1	1	1	1	13	40	135	250
T <sub>16</sub>	1	1	1	1	19	40	135	250
T <sub>17</sub>	-2	0	0	0	10	30	120	200
T <sub>18</sub>	2	0	0	0	22	30	120	200
T <sub>19</sub>	0	-2	0	0	16	10	120	200
T <sub>20</sub>	0	2	0	0	16	50	120	200
T <sub>21</sub>	0	0	-2	0	16	30	90	200
T <sub>22</sub>	0	0	2	0	16	30	150	200
T <sub>23</sub>	0	0	0	-2	16	30	120	100
T <sub>24</sub>	0	0	0	2	16	30	120	300
T <sub>25</sub>	0	0	0	0	16	30	120	200
T <sub>26</sub>	0	0	0	0	16	30	120	200
T <sub>27</sub>	0	0	0	0	16	30	120	200
T <sub>28</sub>	0	0	0	0	16	30	120	200
T <sub>29</sub>	0	0	0	0	16	30	120	200
T <sub>30</sub>	0	0	0	0	16	30	120	200

FMC, feed moisture content, DPF, defatted peanut flour, DHT, die head temperature.

Kolkata, India). Prior to use the extruder, it was kept in the running condition without feeding the material. It is necessary for removal material residue deposited in the barrel assembly. After cleaning of barrel, the heating system of twin screw extruder was kept in the on condition for a duration till the required temperatures were attained at different sections. The round hole die of 3 mm diameter

was used for preparing the extruded product. 300 gram of composite flour prepared by blending of corn flour and defatted peanut flour was fed and extrusion was carried out with different processing variables. The cutter speed was adjusted appropriately. The extruded product was collected in a tray and dried using laboratory tray drier at 60 °C temperature for 1 hour to reduce moisture



content up to 2-3% (w.b.) from product. Then product was packed in zipped lock plastic bags and put under storage at room temperature for future analysis.

### Physical, functional and mechanical properties

The ratio of diameter of extruded and the diameter of die was used to express the expansion of extruded (Fan *et al.* 1996). The Water Solubility Index (WSI) and Water Absorption Index (WAI) were measured using a technique developed for cereals (Ding *et al.* 2006). The Water Holding Capacity (WHC) was calculated using the formula given by Deshpande and Poshadri (2011).

### STATISTICAL ANALYSIS

The statistical analysis of the experimental data was carried out to observe the effect of selected process parameters on the various responses. The obtained data were subjected to analyse for graphical representation, analysis of variance (ANOVA) and multiple regression using the software Design Expert version 10 (Anderson and Whitcomb, 2005). The three-dimensional (3D) response surface plot were generated by keeping one variable constant at the centre point and varying the other two variables within the experimental range. The effect and regression coefficients of individual linear, interaction terms and quadratic terms were determined from the ANOVA table. The significance of all the terms in the polynomial equation was judged statistically by computing the F-value at a probability (p) value of 0.001, 0.01 and 0.05.

## RESULTS AND DISCUSSION

### Proximate composition of raw materials

The biochemical characteristics of the corn flour and defatted peanut flour selected for the study are presented in the Table 3.

### Effect of feed moisture content, defatted peanut flour, die head temperature and screw speed on response variables

The treatment-wise data regarding various physical and functional characteristics of extruded products prepared by blending of DPF and corn flour is presented in the Table 4.

### Expansion ratio

The effect of feed moisture content, defatted peanut flour, die head temperature and screw speed on expansion ratio of extrudates is presented in the Table 4. while the response surface plots are shown in the Fig.1. ER increased with an increase in the DPF and FMC up to its minimum level *i.e.* 10% and 10% (w.b.), respectively. Similarly, another probability for increased in expansion ratio with increase the FMC up to its maximum level *i.e.* 22 % (w.b.) and DPF up to its minimum level *i.e.* 10%. Further, the ER increased with an increase the DHT up to its maximum level *i.e.* 150 °C and FMC is up to 10% (w.b.). The ER also increased with an increase the screw speed up to its maximum level *i.e.* 300 rpm and FMC up to its minimum level *i.e.* 10% (w.b.). It can also be observed that the ER increased with an increase in the DPF up to its minimum level *i.e.* 10% and DHT up to its maximum level *i.e.* 150 °C. The interaction of DPF and screw speed showed that the ER increased with increase the screw speed up to its maximum level *i.e.* 300 rpm and DPF up to its minimum level *i.e.* 10 %. DHT and screw speed at its maximum level *i.e.* 150 °C and 300 rpm, gave the highest ER of 2.30 mm/mm. Banerjee *et al.* (2003) also studied expansion ratio decreased with increase in moisture content. Ding *et al.* (2004) also studied higher barrel temperature increased the extrudate expansion. Liu *et al.* (2000) observed screw speed had no significant effect on expansion ratio. Suknark *et al.* (1997) studied as amylase content of starch increased, the expansion ratio increased.

**Table 3:** Biochemical characteristics of corn flour and defatted peanut flour.

Sl. No.	Characteristic	Corn flour	Defatted peanut flour
1	Moisture content % (w.b.)	8.62±0.23	5.64±0.09
2	Carbohydrate (%)	72.35±2.73	23.59±0.57
3	Protein (%)	9.55±0.39	61.98±0.77
4	Fat (%)	5.71±0.27	3.96±0.19
5	Ash (%)	1.64±0.04	4.76±0.17

**Table 4:** Physical and functional characteristics of extruded product prepared by blending of defatted peanut flour and corn flour

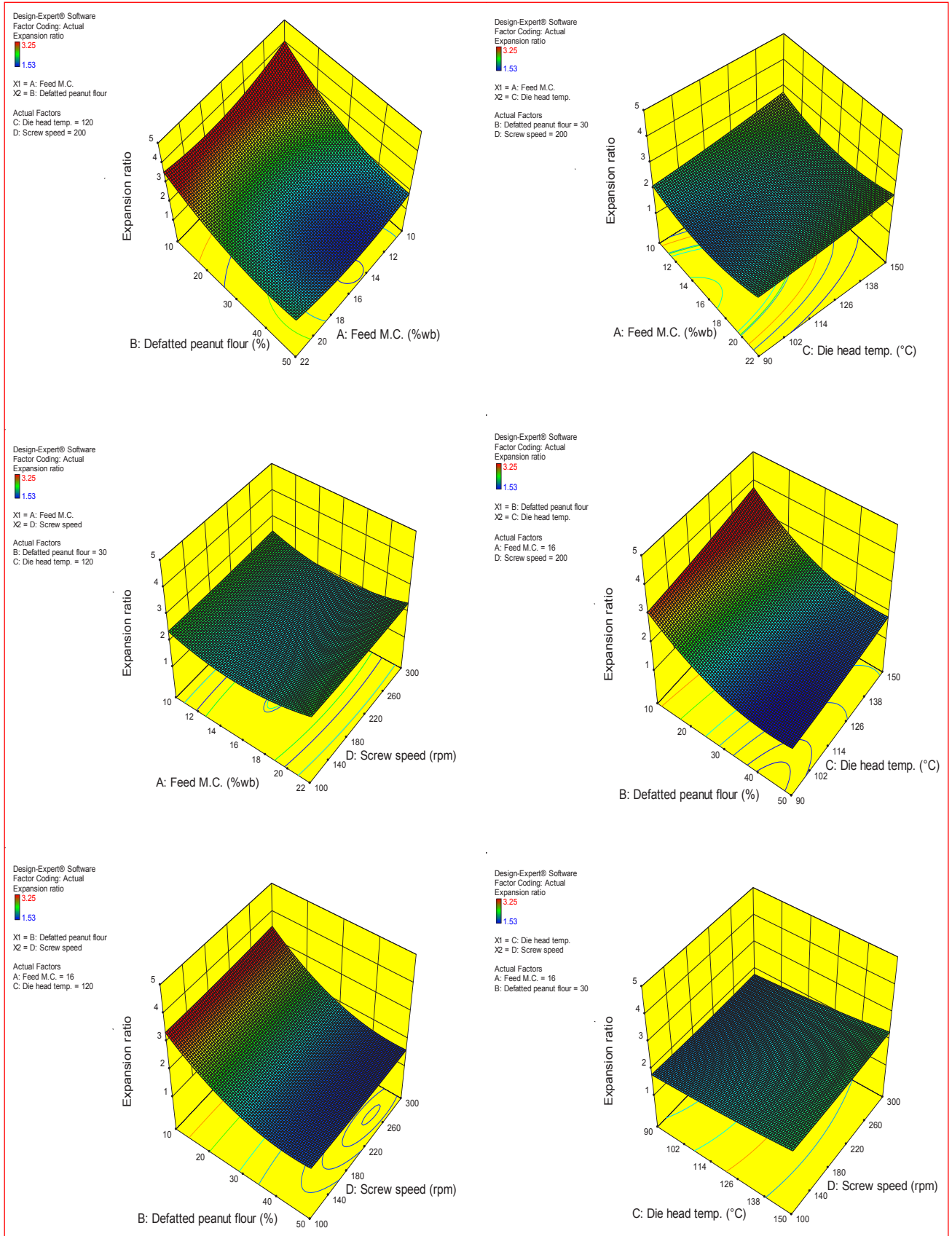
Treatment No.	Independent variable				Responses			
	FMC ( $X_1$ ) % (w.b.)	DPF ( $X_2$ ) (%)	DHT ( $X_3$ ) (°C)	Screw speed ( $X_4$ ) (rpm)	ER (mm/mm)	WSI (%)	WAI (g/g)	WHC (%)
T <sub>1</sub>	13	20	105	150	2.59	10.55	5.25	401.21
T <sub>2</sub>	19	20	105	150	2.54	4.46	5.23	388.56
T <sub>3</sub>	13	40	105	150	1.79	11.23	4.26	357.41
T <sub>4</sub>	19	40	105	150	1.69	7.48	4.62	375.10
T <sub>5</sub>	13	20	135	150	2.98	18.02	5.79	482.32
T <sub>6</sub>	19	20	135	150	2.61	13.85	5.61	475.65
T <sub>7</sub>	13	40	135	150	1.80	16.50	4.22	402.57
T <sub>8</sub>	19	40	135	150	1.80	12.13	4.34	389.53
T <sub>9</sub>	13	20	105	250	2.80	8.60	5.46	412.32
T <sub>10</sub>	19	20	105	250	2.58	5.88	6.58	417.21
T <sub>11</sub>	13	40	105	250	1.76	12.11	4.42	389.65
T <sub>12</sub>	19	40	105	250	1.71	8.65	4.09	377.68
T <sub>13</sub>	13	20	135	250	3.05	18.85	6.11	498.56
T <sub>14</sub>	19	20	135	250	2.67	14.52	5.73	465.23
T <sub>15</sub>	13	40	135	250	1.89	14.00	4.83	378.65
T <sub>16</sub>	19	40	135	250	1.87	11.66	5.05	407.21
T <sub>17</sub>	10	30	120	200	2.10	13.27	3.14	399.65
T <sub>18</sub>	22	30	120	200	2.54	10.44	4.52	372.32
T <sub>19</sub>	16	10	120	200	3.25	5.38	5.80	428.70
T <sub>20</sub>	16	50	120	200	1.65	8.17	3.98	373.73
T <sub>21</sub>	16	30	90	200	1.53	9.34	4.83	372.12
T <sub>22</sub>	16	30	150	200	2.35	18.37	5.24	501.23
T <sub>23</sub>	16	30	120	100	2.04	7.46	6.21	456.36
T <sub>24</sub>	16	30	120	300	1.81	11.25	5.21	445.10
T <sub>25</sub>	16	30	120	200	1.69	7.97	4.66	406.65
T <sub>26</sub>	16	30	120	200	2.12	7.10	3.87	431.27
T <sub>27</sub>	16	30	120	200	2.00	13.07	4.43	413.93
T <sub>28</sub>	16	30	120	200	2.04	10.11	4.48	412.11
T <sub>29</sub>	16	30	120	200	1.93	9.07	4.47	436.05
T <sub>30</sub>	16	30	120	200	1.95	9.76	4.39	426.56
Control	13	—	135	250	2.82	4.78	5.22	435.93

ER, expansion ratio, WSI, water solubility index, WAI, water absorption index, WHC, water holding capacity.

### Water Solubility Index (WSI)

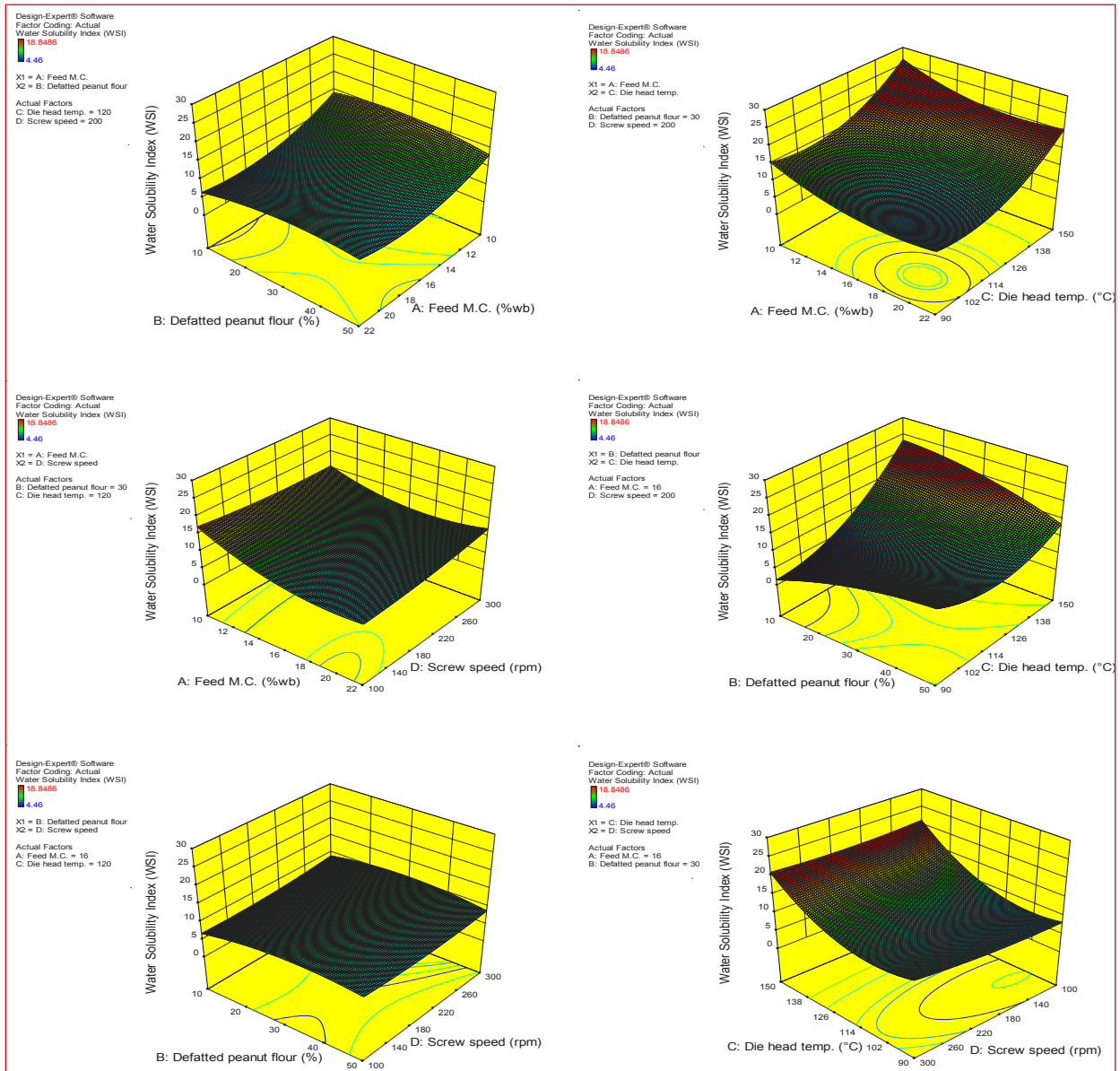
Fig. 2 represents the effect of feed moisture content, defatted peanut flour level, die head temperature and screw speed on water solubility Index. The highest WSI (15.74%) was observed for the interaction of DPF at 30% and FMC at 10%. WSI was observed to be increased up to 23.01% with an increase the DHT up to its maximum level *i.e.* 150 °C and FMC up to its minimum level *i.e.* 10 % (w.b.). The interaction effect of FMC and screw speed showed that the WSI increased up to 17.18% with an increase in the FMC and screw speed up to its minimum level *i.e.* 10% (w.b.) and 100 rpm, respectively. WSI increased with an increased in the

DHT up to its maximum level *i.e.* 150 °C and DPF up to its minimum level *i.e.* 10%. At this interaction WSI was expected to be increased up to 23.31%. WSI was reported to be maximum (10.84%) for the interaction of DPF (30%) and screw speed (300 rpm). For the interaction of DHT and screw speed, the WSI was increased (21%) with an increase in DHT and screw speed up to its maximum level *i.e.* 150 °C and 300 rpm, respectively. Kadan *et al.* (2003) also reported WSI increased with an increase in extrusion temperature. Ding *et al.* (2004) also evaluated increasing feed moisture content results lower WSI.



**Fig. 1:** Effect of feed moisture content, defatted peanut flour, die head temperature and screw speed on expansion ratio





**Fig. 2:** Effect of feed moisture content, defatted peanut flour, die head temperature and screw speed on water solubility index

**Table 5:** Constraints, criteria and output for numerical optimization of extruded snack food

Variables					
Constraint	Goal		Importance	Optimum value	
Feed moisture content % (w.b.)	In the range		3	13	
Defatted peanut flour (%)	Maximum		3	26	
Die head temperature (°C)	In the range		3	135	
Screw speed (rpm)	In the range		3	250	
Responses					
Constraint	Goal	Importance	Predicted value	Experimental value	Deviation (%)
Expansion ratio (mm/mm)	Maximum	3	2.51	2.26	9.96
Water solubility index (%)	Maximum	3	16.47	16.51	-0.243
Water absorption index (g/g)	Maximum	3	5.24	4.27	18.51
Water holding capacity (%)	Maximum	3	467.38	473.44	-1.30



### Water Absorption Index (WAI)

The effect of feed moisture content, defatted peanut flour, die head temperature and screw speed on WAI is graphically presented in the Fig. 3. From the graphs it could be observed that the WAI was increased up to 6.24 g/g with an increase in the DPF level up to its minimum level *i.e.* 10% and FMC up to 18% (w.b.). The interaction of DHT (150°C) and FMC (15.80%) gave the maximum WAI of 5.38 g/g. The WAI was increased up to 6.04 g/g with an increase in the screw speed up to its maximum level *i.e.* 300 rpm and FMC up to 19% (w.b.). The DPF with its minimum level of 10% and DHT with its maximum level of 150°C increased the WAI up to 7.09 g/g. The interaction of DPF and screw speed showed that WAI was increased with increase the DPF up to its minimum level *i.e.* 10% and screw speed up to its maximum level *i.e.* 300 rpm. At this interaction of DPF and screw speed, the WAI increased the up to 7.97 g/g and 7.29 g/g, respectively. The interaction of DHT (150°C) and screw speed (300 rpm) gave the highest WAI of 7.88 g/g. Kadan *et al.* (2003) reported the rise in the WAI with an increase in extrusion temperature. Ding *et al.* (2004) observed that increasing feed moisture content results higher WAI. Pardhi *et al.* (2019) investigated the increase in screw speed resulted in lower WAI. Singh *et al.* (2016) also studied higher extrusion temperature increased WAI. Stojceska *et al.* (2009) that WAI increased with increasing die temperature. Water absorption index decreased with increase in moisture content, which may be attributed to the reduction of elasticity of dough through plasticization of melt at higher moisture content and increase in increase in temperature probably due to increased dextrinization of at higher temperature.

### Water Holding Capacity (WHC)

It could be observed from the Fig. 4 that the WHC was maximum (449.35%) for the DPF level of 10% and FMC up to 14.19% (w.b.). Response graph shows that the interaction of DHT at 150°C and FMC at 15.33% increased the WHC up to 487.03%. WHC also increased with an increase in the screw speed up to its maximum level *i.e.* 300 rpm and FMC up to 15.4% (w.b.) and gave the maximum WHC of 451.61% at this interaction. The positive impact of interaction of DPF and DHT on WHC

was observed up to 10% and 150°C, respectively which yielded the WHC of 566.22%. The interaction of DPF at 10% and screw speed at 300 rpm yielded the highest WHC of 479.86%. Similarly, the WHC was found to be increased up to 526.84% for the interaction of DHT at 150°C and screw speed at 100 rpm. For all the above conditions further rise the independent variables decreased the response variables. Banerjee *et al.* (2003) also reported the rise in the WHC with an increase in the moisture content as well as temperature.

### Empirical models

The derived model, giving the empirical relation between the response variables and test variables in coded units, was obtained as under:

$$ER = 1.96 - 0.01 * A - 0.45 * B + 0.12 * C + 0.00 * D + 0.05 * AB - 0.02 * AC - 0.01 * AD - 0.02 * BC - 0.01 * BD + 0.00 * CD + 0.11 * A^2 + 0.14 * B^2 + 0.01 * C^2 + 0.01 * D^2$$

$$WSI = 9.51 - 1.54 * A + 0.19 * B + 2.86 * C + 0.32 * D + 0.21 * AB + 0.05 * AC + 0.35 * AD - 1.31 * BC - 0.12 * BD - 0.19 * CD + 0.81 * A^2 - 0.47 * B^2 + 1.31 * C^2 + 0.18 * D^2$$

$$WAI = 4.38 + 0.15 * A - 0.57 * B + 0.11 * C + 0.04 * D - 0.01 * AB - 0.08 * AC + 0.02 * AD + 0.02 * BC - 0.07 * BD + 0.04 * CD - 0.10 * A^2 + 0.17 * B^2 + 0.20 * C^2 + 0.37 * D^2$$

$$WHC = 421.10 - 3.38 * A - 23.88 * B + 26.62 * C + 2.15 * D + 4.31 * AB - 1.40 * AC + 0.18 * AD - 14.02 * BC - 1.06 * BD - 4.69 * CD - 9.61 * A^2 - 5.80 * B^2 + 3.07 * C^2 + 6.58 * D^2$$

Where, *A*, *B*, *C* and *D* are the coded factors of feed moisture content, defatted peanut flour, die head temperature and screw speed, respectively.

### Optimization and validation of process variables

The optimum condition for the development of extruded product by blending of corn flour and defatted peanut flour was determined by the numerical optimization technique, using Design Expert software version 10 (State-Ease Inc., Minneapolis, MN, USA). The main criteria applied for constraints optimization in the study were given in the Table 5. Accordingly, the goals that were set for variables and responses to obtain

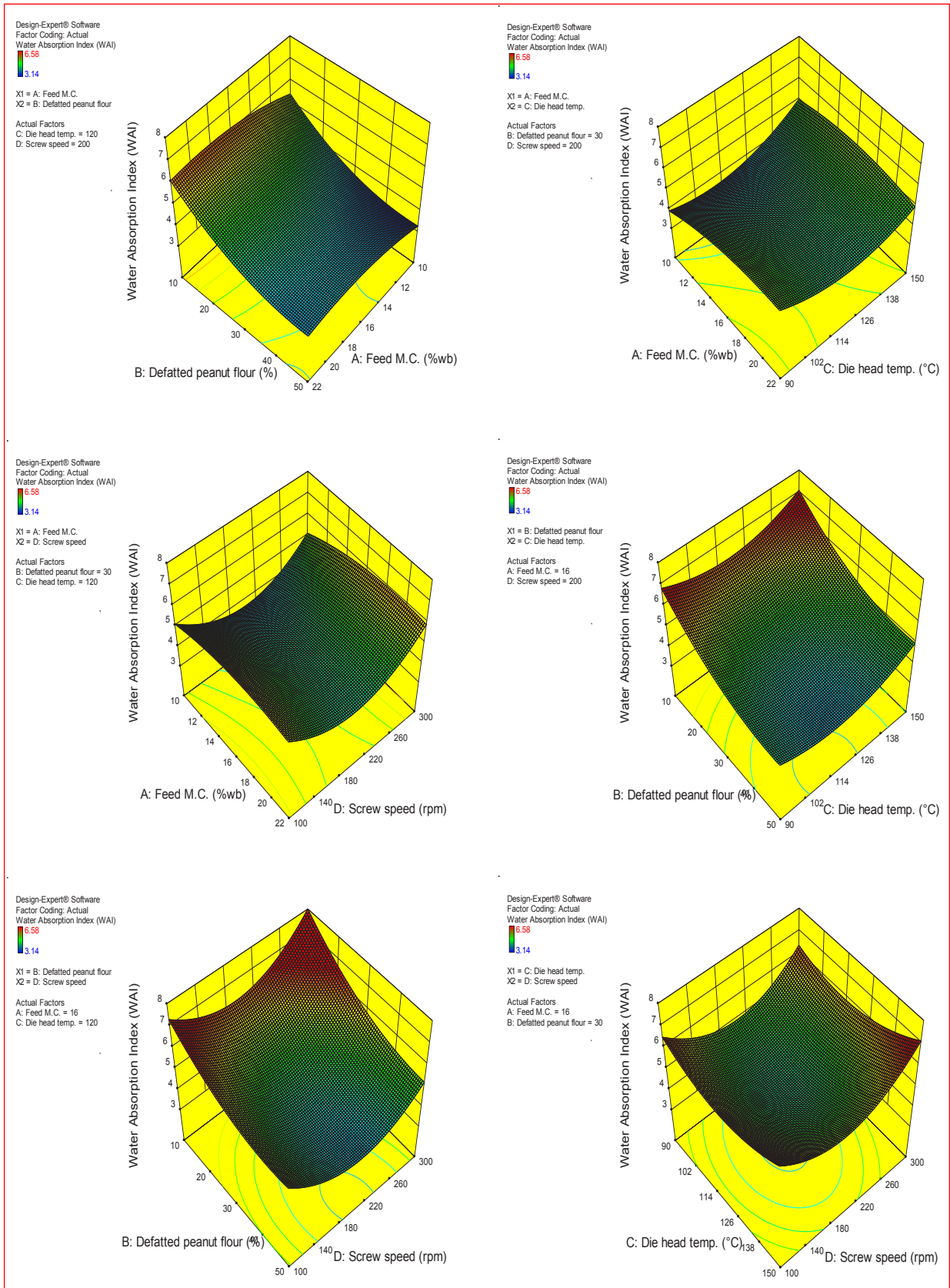


Fig. 3: Effect of feed moisture content, defatted peanut flour, die head temperature and screw speed on water absorption index

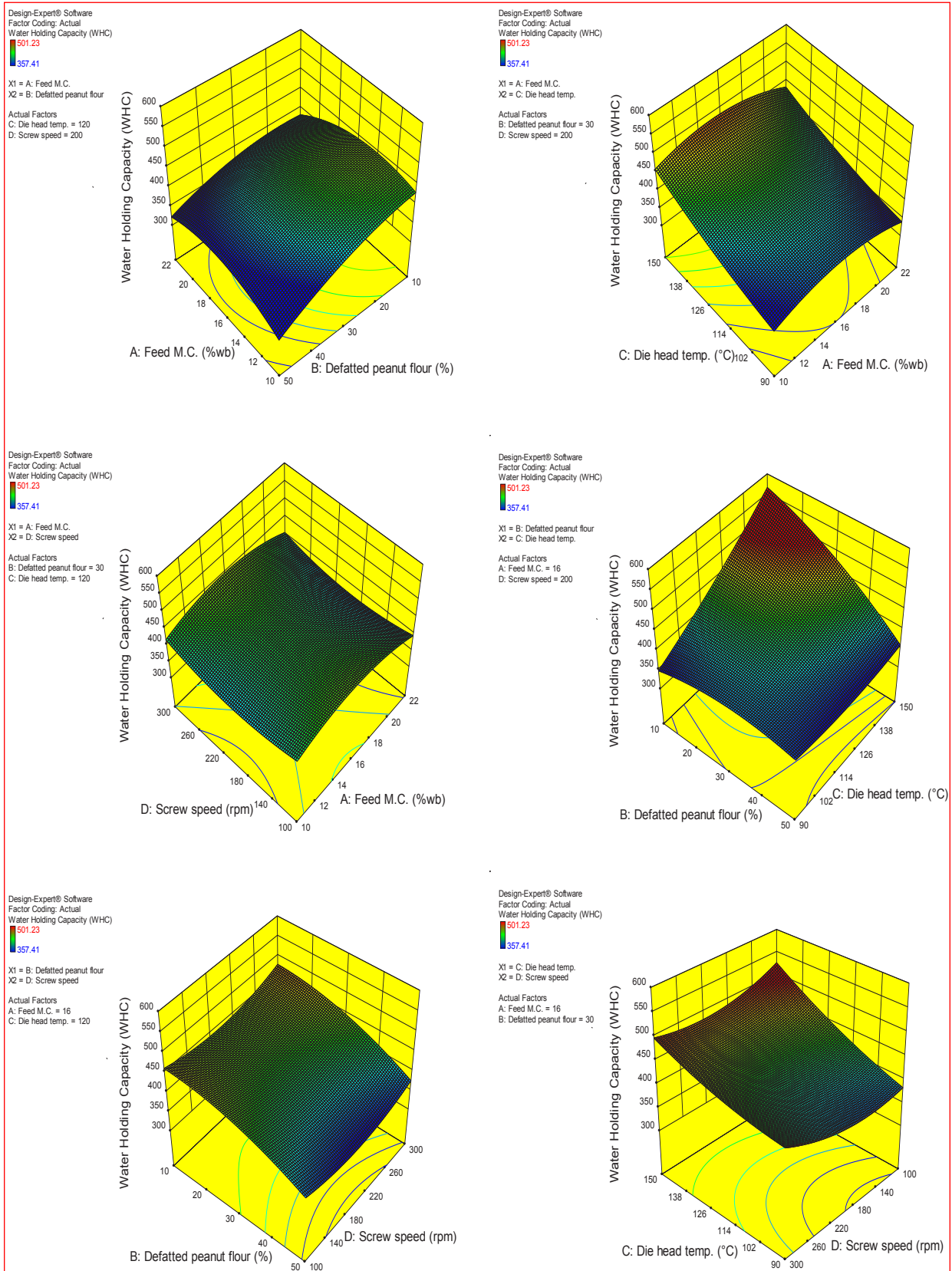


Fig. 4: Effect of feed moisture content, defatted peanut flour, die head temperature and screw speed on water holding capacity



the best combination are illustrated in the Table 5. All the independent variables and responses were given equal importance, *i.e.* 3, during optimization process. Under these constraints, the optimum treatment conditions were found to be, feed moisture content 13% (w.b.), defatted peanut flour 26%, die temperature 135 °C and screw speed 250 rpm. Using these optimized conditions the experiment was repeated to find the variation in the different response variables so as to check the validity of predicted model. The results revealed that the experimental values of conducted experiments were very close to the optimized predicted values (Table 5). This implied that there was a high degree of fit between the observed and predicted values from the regression models and each model was quite accurate in prediction. The closeness of the observed and predicted responses indicated the validity of developed model.

## CONCLUSION

It could be concluded from the study that the defatted peanut flour can suitably be used for the extrusion cooking process only if mixed properly with corn flour due to its high protein content, which restricts product gelatinization and limits the product expansion during extrusion cooking. Defatted peanut flour can be used as functional ingredient in the production of extruded snack products due to its high protein content and nutritional value. Among the different experimental conditions, the optimized condition was obtained as 13% feed moisture content, 26% defatted peanut flour, 135°C die head temperature, 250 rpm screw speed, 100°C barrel temperature and 60°C feed temperature for the production of extruded product with better expansion ratio and functional properties.

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# Effect of Blanching on the Quality of Green Peas During Freezing

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## ABSTRACT

Green peas are a very good source of protein, dietary fibre, vitamin K, vitamin B<sub>1</sub>, vitamin C, vitamin B<sub>2</sub>, vitamin B<sub>6</sub>, folate, manganese, phosphorus, copper, niacin, molybdenum, zinc, magnesium, iron and potassium. Freezing is one of the oldest and most widely used methods of food preservation, which allows preservation of taste, texture and nutritional value in foods better than other methods. Blanching is a thermal process designed to inactivate the enzymes responsible for generating the off-flavours and off-colours. Fresh green peas were blanched for various temperature (70, 80, 90 and 100 °C) and time (1, 2 and 3 min) in 4% maltose solution and stored in plastic zip lock bag (50 micron) at -18 °C for 6 months of storage. At 45 days intervals frozen peas were analyzed for biochemical and sensory parameters. Treatment 80 °C temperature and 2 min blanched in 4% maltose solution was observed to be best treatment amongst all treatments considering firmness, protein, carbohydrate, ascorbic acid, chlorophyll content and overall acceptability of frozen green peas at the end of storage period.

## HIGHLIGHTS

- ❶ Blanching of green peas.
- ❷ Freezing of green peas.
- ❸ Standardization of freezing process for storage of green peas.

**Keywords:** Peas, Blanching, Freezing, Firmness, Biochemical properties

Vegetables are important food and highly beneficial for the maintenance of health and prevention of diseases. They are valued for their high carbohydrate, vitamin, mineral and fibre contents. It is a cool-season crop grown in many parts of the world; planting can take place from winter to early summer depending on location. The average pea weighs between 0.1 and 0.36 g. The immature peas (and in snow peas the tender pod as well) are used as a vegetable, fresh, frozen or canned; varieties of the species typically called field peas are grown to produce dry peas like the split pea shelled from the matured pod.

Blanching is a thermal process designed to inactivate the enzymes responsible for generating the off-flavours and off-colours. All living tissue contains

enzymes. Apart of enzyme inactivation, blanching of vegetables prior to freezing has several advantages. The advantages include stabilization of texture, flavour and nutritional quality, destruction of microorganisms, reduces drying time, expulses air from the tissue and better retains minerals and acids (Cano 1996). The rate of enzyme action is greatly reduced by refrigeration. However, even at -18 °C, enzyme action is still sufficiently rapid to change the flavour of unblanched vegetables in a few weeks (Tresaler, 1938). The ascorbic acid contents

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of water blanched green beans were higher than steamed blanched samples and convection oven blanched green beans were found to be lowest in ascorbic acid and chlorophyll contents as reported by Muftugil (1986). Freezing is used to maintain product quality over long storage and results in a slower rate of most deteriorative reactions such as senescence, enzymatic decay, chemical decay and microbial growth. However, freezing does not prevent off-flavour development, color and texture deterioration in frozen vegetables because enzymes systems remain active even at sub-zero temperature (Rodriguez-Sanoa *et al.* 1995). In order to prevent enzymatic reactions during freezing, most vegetables must first be blanched. Blanching can be carried out by different methods, but hot water blanching is most widely used techniques for this purpose. Neri *et al.* (2011) used trehalose and maltose in the blanching solution and found that the blanching solution reduced the solute loss while increasing the water loss.

Freezing has been successfully employed for the long-term preservation of many foods, providing a significantly extended shelf life. The process involves lowering the product temperature generally to  $-18^{\circ}\text{C}$  or below. Blanching is must for almost all vegetables to be frozen. Blanching is thermal processing needs optimization of blanching parameter on better quality of frozen pea.

## MATERIALS AND METHODS

The pods of green peas were procured from Marketing Yard, Junagadh. Damaged, undeveloped and thin pods of pea were removed. Shelling the green peas from the pods was carried out manually. Only matured, round shaped, dark green and uniform sized were selected for experimental work. For blanching 4% maltose solution was prepared by dissolving 4 g maltose powder in 100 ml distilled water. The solution of 4% maltose was boiled at different temperature and time combination in glass beaker using heating mantle. The 200 g of green peas were blanched in 4%, 500ml maltose solution heated in the beaker after obtaining the temperature ( $70^{\circ}\text{C}$ ,  $80^{\circ}\text{C}$ ,  $90^{\circ}\text{C}$  and  $100^{\circ}\text{C}$ ) for different time (1, 2 and 3 min) with one control in distilled water blanched ( $80^{\circ}\text{C}$  and 2 min time). Immediate cooling is necessary after blanching is to avoid overheating. The cooling process of blanched peas was carried

out by immersing the peas in the container of cold water for 10 min. ( $20^{\circ}\text{C}$  temperature). Surface water is removed at room temperature. The blanched peas were packed in plastic bags. After filling process, bags of different treatments were labelled as per its treatment order. Finally, labelled bags of different treatments were stored at  $-18^{\circ}\text{C}$  temperature for storage in deep freezer (Blue Star CHF500 Capacity 484 Lit.). The quality evaluation of these bags was carried out on the basis of various biochemical parameters at an interval of 45 days of storage in frozen condition up to 6 month storage.

After blanching as well as during frozen storage biochemical parameters was measured for all 14 treatments as per following methods. The firmness of peas was measured by using texture analyzer (Model: TA.XT Plus, Cap. 50 kg) at Food Testing Laboratory, Biochemistry Department, JAU, Junagadh. Protein content of peas was estimated as per the method suggested by Lowry *et al.* (1951). Carbohydrate, chlorophyll, ascorbic acid was determined as per the method suggested by Sadasivam and Manickam (1996). The sensory evaluation of green peas was carried out by 9-point hedonic scale method described by Rangana (2000).

## RESULTS AND DISCUSSION

### Effect of blanching temperature and time on firmness of peas

The treatment wise effect of blanching temperature and time on firmness of peas is shown graphically in Fig. 1. It is evident from the figure that firmness of peas decreased with increase in storage period in all treatments. The decrease in firmness during storage may be due to cellular disintegration which leads to membrane permeability. Similar trend was also observed by Halpin and Lee, 1987 in case of firmness of peas. The maximum firmness of peas (416.60 g) was found for the treatment  $T_5$  ( $80^{\circ}\text{C}$  temperature and 2 min) followed by treatment  $T_4$  ( $80^{\circ}\text{C}$  temperature and 1 min) after 180 days of storage period (Table 1). The minimum firmness of peas (363.13 g) was found for the treatment  $T_{14}$  (unblanched) at end of 180 days of storage period. The minimum percentage decrease (6.83%) was found in treatment  $T_{11}$  ( $100^{\circ}\text{C}$  temperature and 2 min) and the maximum percentage decrease (32.70%) was found in treatment  $T_{14}$  (unblanched). Firmness



**Table 1:** Effect of different treatments on biochemical and sensory parameters of peas during freezing

Sl. No.	Treatment	After 180 days of storage					
		Firmness	Protein	Carbohydrate	Chlorophyll	Ascorbic Acid	Overall Acceptability
1	T <sub>1</sub> (70 °C and 1 min)	401.16 (- 22.47)	5.78 (+ 4.29)	13.98 (+ 13.41)	0.88 (- 30.26)	29.68 (- 10.51)	4.45 (- 41.46)
2	T <sub>2</sub> (70 °C and 2 min)	399.99 (- 18.31)	5.73 (+ 4.05)	13.90 (+ 13.41)	0.88 (- 28.57)	29.14 (- 7.98)	4.00 (- 46.64)
3	T <sub>3</sub> (70 °C and 3 min)	407.09 (- 12.18)	5.65 (+ 4.05)	13.87 (+ 13.57)	0.93 (- 21.35)	28.39 (- 7.06)	5.73 (- 20.40)
4	T <sub>4</sub> (80 °C and 1 min)	416.59 (- 14.83)	5.72 (+ 4.19)	13.92 (+ 13.42)	0.99 (- 18.90)	28.57 (- 12.27)	5.64 (- 24.82)
5	T <sub>5</sub> (80 °C and 2 min)	416.60 (- 10.55)	5.64 (+ 4.17)	13.86 (+ 13.58)	1.11 (- 6.96)	29.92 (- 4.98)	6.45 (- 15.16)
6	T <sub>6</sub> (80 °C and 3 min)	401.54 (- 9.22)	5.62 (+ 4.45)	13.70 (+ 13.63)	1.09 (- 8.17)	27.37 (- 8.97)	6.00 (- 17.79)
7	T <sub>7</sub> (90 °C and 1 min)	398.45 (- 14.18)	5.68 (+ 4.60)	13.78 (+ 13.67)	1.04 (- 12.39)	29.70 (- 7.64)	5.36 (- 29.52)
8	T <sub>8</sub> (90 °C and 2 min)	378.98 (- 13.25)	5.53 (+ 4.04)	13.58 (+ 13.80)	1.03 (- 10.98)	28.41 (- 8.23)	6.10 (- 18.67)
9	T <sub>9</sub> (90 °C and 3 min)	381.34 (- 8.89)	5.48 (+ 3.89)	13.30 (+ 14.15)	1.04 (- 6.13)	26.52 (- 9.33)	5.50 (- 28.50)
10	T <sub>10</sub> (100 °C and 1 min)	388.09 (- 10.08)	5.54 (+ 4.46)	13.08 (+ 14.33)	1.06 (- 9.90)	28.36 (- 9.31)	5.30 (- 26.44)
11	T <sub>11</sub> (100 °C and 2 min)	380.17 (- 6.83)	5.44 (+ 4.30)	12.80 (+ 14.88)	0.93 (- 15.28)	25.07 (- 13.90)	5.70 (- 22.94)
12	T <sub>12</sub> (100 °C and 3 min)	363.14 (- 8.47)	5.44 (+ 4.52)	12.59 (+ 15.13)	0.95 (- 8.03)	25.29 (- 7.37)	5.50 (- 25.67)
13	T <sub>13</sub> **	384.12 (- 15.96)	5.59 (+ 4.52)	13.30 (+ 14.13)	1.02 (- 12.68)	28.07 (- 8.70)	6.00 (- 21.03)
14	T <sub>14</sub> (unblanched)	363.13 (- 32.70)	5.87 (+ 4.48)	14.79 (+ 12.54)	0.80 (- 39.09)	29.34 (- 27.29)	4.64 (- 36.51)

\* Data in brackets shows percentage increase (+) or decrease (-) during entire storage period.

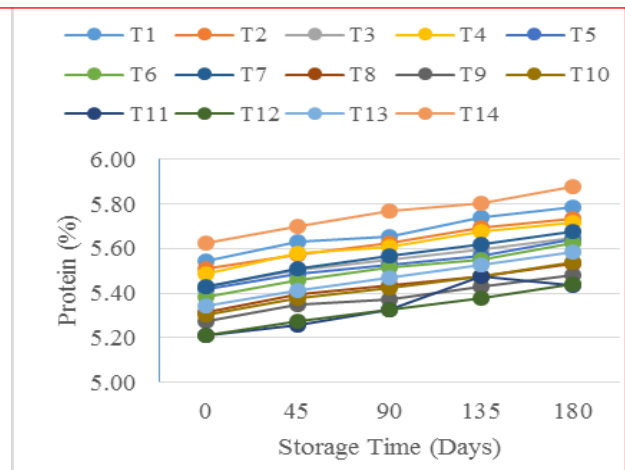
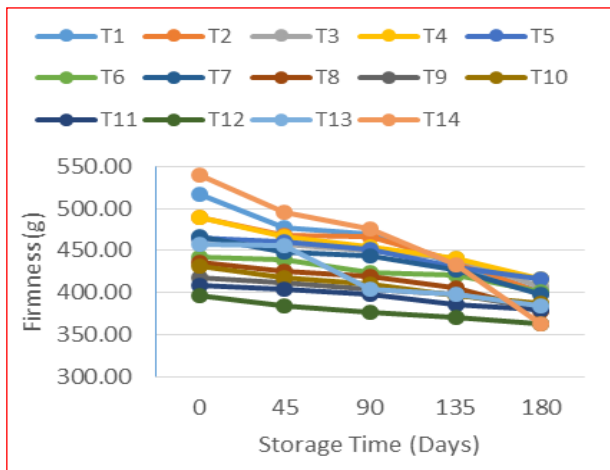
\*\* T<sub>13</sub> = 80 °C temperature and 2 min blanching time in distilled water without 4 % maltose solution.

generally decreased as blanching temperature increases. Similar trend was also observed by Halpin and Lee, 1987 in case of firmness of peas.

The effect of blanching temperature and time on firmness of peas is presented in Table 2. For the blanching temperature, the maximum firmness of peas (411.58 g) was obtained for 80 °C and minimum firmness of peas (377.13 g) was obtained for 100 °C at end of 180 days of storage period. The minimum percentage decrease (8.49%) was found for the temperature T<sub>4</sub> (100 °C temperature) and the maximum percentage decrease (17.84%) was found for the temperature T<sub>1</sub> (70 °C temperature). The statistical analysis of the data showed that the

effect of blanching temperature on the firmness of peas was found significant at the end of storage period at 5% level of significance.

Effect of blanching time showed that the maximum firmness of peas (401.07 g) was obtained for the blanching time of 1 min and minimum (388.28 g) was obtained 3 min of blanching time at the end of 180 days of storage period. The minimum percentage decrease in firmness of peas (9.76%) was found when the blanching time was 3 min and the maximum percentage decrease in firmness of peas (15.74%) was found for the blanching time of 1 min. The statistical analysis of blanching time showed that the firmness of peas was significant at



**Fig. 1:** Effect of storage time on firmness content of frozen peas

**Fig. 2:** Effect of storage time on protein content of frozen peas

the end of storage period at 5% level of significance. The interaction effect of blanching temperature and blanching time on firmness of peas was observed to be significant at the end of storage period at 5% level of significance (Table 2).

### Effect of blanching temperature and time on protein content of peas

The treatment wise effect of blanching temperature and time on protein content of peas is shown graphically in Fig. 2. It is evident from the figure that protein content of peas increased with increase in storage period in all treatments. The maximum protein content of peas (5.87 %) was found for the treatment  $T_{14}$  (unblanched) after 180 days of storage period. The minimum protein content of peas (5.44 %) was found for the treatment  $T_{11}$  (100 °C temperature and 2 min) and treatment  $T_{12}$  (100 °C temperature and 3 min) at end of 180 days of storage period (Table 1). The increase in protein content during storage might be due to moisture losses during storage. The maximum percentage increase (4.60%) was found in treatment  $T_7$  (90 °C temperature and 1 min) and the minimum percentage increase (3.89%) was found in treatment  $T_9$  (90 °C temperature and 3 min).

The effect of blanching temperature and time on protein content of peas is presented in Table 2. For the blanching temperature, the maximum protein content of peas (5.72 %) was obtained for 70 °C and minimum protein content of peas (5.47 %) was obtained for 100 °C at end of 180 days of storage period. The maximum percentage increase (4.39%)

was found for the 100 °C temperature and the minimum percentage increase (4.12%) was found for the 90 °C temperature. The statistical analysis of the data showed that the effect of blanching temperature on the protein content of peas was found significant at the end of storage period at 5% level of significance.

Effect of different blanching times showed that the maximum protein content of peas (5.68 %) was obtained for the blanching time of 1 min and minimum (5.55 %) was obtained 3 min of blanching time at the end of 180 days of storage period. The maximum percentage increase in protein content of peas (4.41%) was found when the blanching time was 1 min and the minimum percentage increase in protein content of peas (4.29%) was found for the blanching time of 2 min. The statistical analysis of blanching time showed that the protein content of peas was found significant at the end of storage period at 5% level of significance. The interaction effect of blanching temperature and blanching time on protein content of peas was observed to be non-significant at the end of storage period at 5% level of significance (Table 2).

### Effect of blanching temperature and time on carbohydrate content of peas

The treatment wise effect of blanching temperature and time on carbohydrate content of peas is shown graphically in Fig. 3. It is evident from the figure that carbohydrate content of peas increased with increase in storage period in all treatments. The maximum carbohydrate content of peas (14.79%)

**Table 2:** Effect of blanching temperature and time on biochemical and sensory parameters of peas

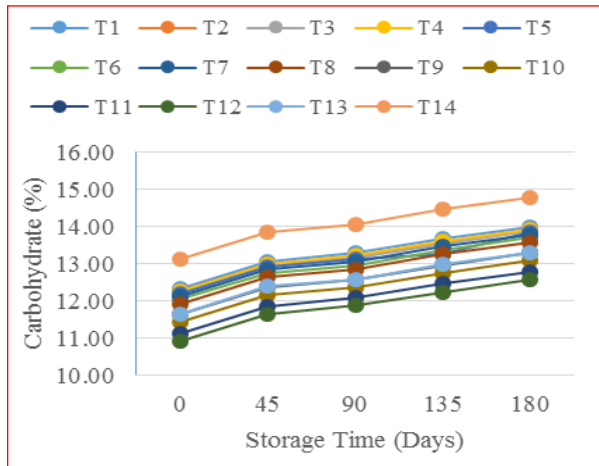
Effect	After 180 days of storage					
	Biochemical and sensory parameters					Overall Acceptability
	Firmness	Protein	Carbohydrate	Chlorophyll	Ascorbic Acid	
<b>Effect of temperature (T)</b>						
T <sub>1</sub> (70 °C)	402.75 (- 17.84)	5.72 (+ 4.19)	13.92 (+ 13.45)	0.90 (- 26.83)	28.74 (- 9.61)	4.73 (- 36.34)
T <sub>2</sub> (80 °C)	411.58 (- 11.63)	5.66 (+ 4.24)	13.83 (+ 13.55)	1.06 (- 11.67)	28.62 (- 8.78)	6.03 (- 19.28)
T <sub>3</sub> (90 °C)	386.26 (- 12.30)	5.56 (+ 4.12)	13.55 (+ 13.87)	1.04 (- 9.57)	28.21 (- 8.37)	5.65 (- 25.66)
T <sub>4</sub> (100 °C)	377.13 (- 8.49)	5.47 (+ 4.39)	12.82 (+ 14.77)	0.98 (- 10.91)	26.24 (- 10.23)	5.50 (- 24.97)
S. Em±	0.78	0.0313	0.1115	0.0172	0.1995	0.0299
CD at 5%	2.29	0.0912	0.3254	0.0501	0.5825	0.0872
<b>Effect of time (t)</b>						
t <sub>1</sub> (1 min)	401.07 (- 15.74)	5.68 (+ 4.41)	13.69 (+ 13.70)	0.99 (- 18.18)	28.83 (- 10.72)	5.18 (- 30.66)
t <sub>2</sub> (2 min)	393.94 (- 12.47)	5.59 (+ 4.29)	13.54 (+ 13.97)	0.99 (- 15.38)	28.14 (- 8.67)	5.57 (- 25.73)
t <sub>3</sub> (3 min)	388.28 (- 9.76)	5.55 (+ 4.32)	13.36 (+ 14.09)	1.00 (- 11.50)	26.89 (- 8.19)	5.68 (- 23.24)
S. Em±	0.68	0.0271	0.0966	0.0149	0.1728	0.0259
CD at 5%	1.98	0.079	NS	NS	0.5044	0.0755
<b>Interaction (T x t)</b>						
S. Em±	1.36	0.0541	0.1931	0.0297	0.3456	0.0518
CD at 5%	3.96	NS	NS	0.0868	1.0089	0.1511
C.V.%	0.60	1.673	2.4723	5.1807	2.1416	1.6363

\* Data in brackets shows percentage increase (+) or decrease (-) during entire storage period.

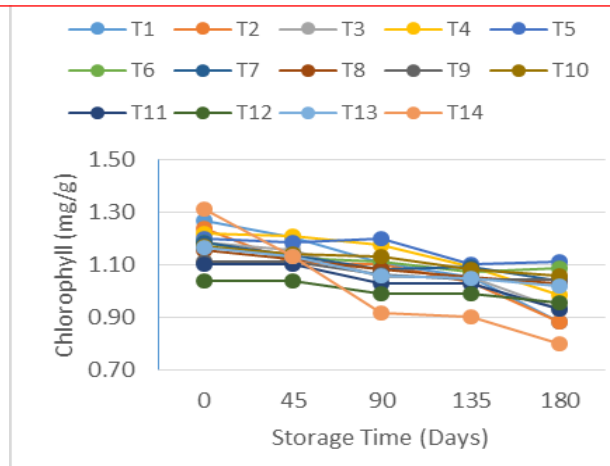
was found for the treatment T<sub>14</sub> (unblanched) after 180 days of storage period. The minimum carbohydrate content of peas (12.59 %) was found for the treatment T<sub>12</sub> (100 °C temperature and 3 min) at end of 180 days of storage period (Table 1). The increase in carbohydrate content during storage may be due to the increase in concentration of organic solutes as a consequence of water losses in the pea. The maximum percentage increase (15.13%) was found in treatment T<sub>12</sub> (100 °C temperature and 3 min) and the minimum percentage increase (12.54%) was found in treatment T<sub>14</sub> (unblanched). The effect of blanching temperature and time on carbohydrate content of peas is presented in Table 2. For the blanching temperature, the maximum carbohydrate content of peas (13.92 %) was obtained for 70 °C and minimum carbohydrate content of peas (12.82 %) was obtained for 100 °C at end of 180 days of storage period. The maximum percentage increase (14.77%) was found for the 100 °C temperature and the minimum percentage increase (13.45%) was found for the 70 °C temperature. The statistical analysis of the data showed that the effect of blanching temperature

on the carbohydrate content of peas was found significant at the end of storage period at 5% level of significance.

Effect of different blanching times showed that the maximum carbohydrate content of peas (13.69 %) was obtained for the blanching time of 1 min and minimum (13.36 %) was obtained 3 min of blanching time at the end of 180 days of storage period. The maximum percentage increase in carbohydrate content of peas (14.09%) was found when the blanching time was 3 min and the minimum percentage increase in carbohydrate content of peas (13.70%) was found for the blanching time of 1 min. The statistical analysis of blanching time showed that the carbohydrate content of peas was non-significant at the end of storage period at 5% level of significance. It is also observed that the carbohydrate decreases as the temperature and blanching time increases. The interaction effect of blanching temperature and blanching time on carbohydrate content of peas was observed to be non-significant at the end of storage period at 5% level of significance (Table 2).



**Fig. 3:** Effect of storage time on carbohydrate content of frozen peas



**Fig. 4:** Effect of storage time on chlorophyll content of frozen peas

### Effect of blanching temperature and time on chlorophyll of peas

The treatment wise effect of blanching temperature and time on chlorophyll of peas is shown in Fig. 4. It is evident from the figure that chlorophyll of peas decreased with increase in storage period in all treatments. The maximum chlorophyll of peas (1.11 mg/g) was found for the treatment T<sub>5</sub> (80 °C temperature and 2 min) after 180 days of storage period. The minimum chlorophyll of peas (0.80 mg/g) was found for the treatment T<sub>14</sub> (unblanched) at end of 180 days of storage period (Table 1). The minimum percentage decrease (6.13%) was found in treatment T<sub>9</sub> (90 °C temperature and 3 min) and the maximum percentage decrease (39.09%) was found in treatment T<sub>14</sub> (unblanched). Decrease in chlorophyll during blanching in water, it diffuses throughout the cell to the blanching medium and is leached out into the blanching media through damaged cell walls (Lee, 1958). According to Heaton *et al.* (1996), decreases in the level of chlorophyll during frozen storage are due to the activity of chlorophyllase. The results are in agreement with the results reported by Bahceci *et al.* (2004) for frozen storage of green beans. Lisiewska *et al.* (2010) also found the content of chlorophyll after 12 months of frozen storage significantly reduced in all samples.

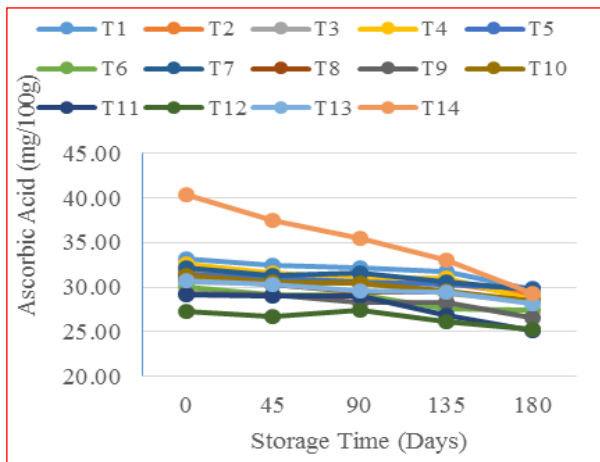
The effect of blanching temperature and time on chlorophyll of peas is presented in Table 2. For the blanching temperature, the maximum chlorophyll of peas (1.06 mg/g) was obtained for 80 °C and minimum chlorophyll of peas (0.90 mg/g) was obtained for 70 °C at end of 180 days of storage

period. The minimum percentage decrease (9.57%) was found for the 90 °C temperature and the maximum percentage decrease (26.83%) was found for the 70 °C temperature. The statistical analysis of the data showed that the effect of blanching temperature on the chlorophyll of peas was found significant at the end of storage period at 5% level of significance.

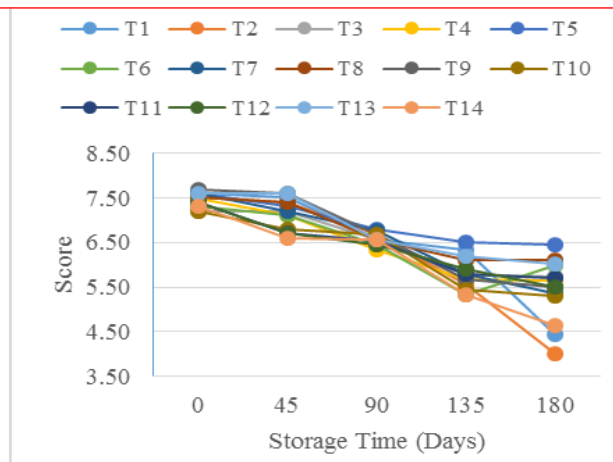
Effect of different blanching times showed that the maximum chlorophyll of peas (1.00 mg/g) was obtained for the blanching time of 3 min and minimum (0.99 mg/g) was obtained 1 min and 2 min of blanching time at the end of 180 days of storage period. The minimum percentage decrease in chlorophyll of peas (11.50%) was found when the blanching time was 3 min and the maximum percentage decrease in chlorophyll of peas (18.18%) was found for the blanching time of 1 min. The statistical analysis of blanching time showed that the chlorophyll of peas was non-significant at the end of storage period at 5% level of significance. The interaction effect of blanching temperature and blanching time on chlorophyll of peas was found significant at the end of storage period at 5% level of significance (Table 2).

### Effect of blanching temperature and time on ascorbic acid of peas

The treatment wise effect of blanching temperature and time on ascorbic acid of peas is shown in Fig. 5. It is evident from the figure that ascorbic acid of peas decreased with increase in storage period in all treatments. The maximum ascorbic



**Fig. 5:** Effect of storage time on ascorbic acid content of frozen peas



**Fig. 6:** Effect of storage time on overall acceptability of frozen peas

acid of peas (29.92 mg/100g) was found for the treatment  $T_5$  (80 °C temperature and 2 min) after 180 days of storage period. The minimum ascorbic acid of peas (25.07 mg/100g) was found for the treatment  $T_{11}$  (100 °C temperature and 2 min) at end of 180 days of storage period (Table 1). The minimum percentage decrease (4.98%) was found in treatment  $T_5$  (80 °C temperature and 2 min) and the maximum percentage decrease (27.29%) was found in treatment  $T_{14}$  (unblanched). Ascorbic acid is lost during water blanching is due to leaching in water and can be expected to occur from thermal degradation and due to the action of ascorbic acid oxidase (Halpin and Lee, 1987). The results are in agreement with the results reported by Bahceci *et al.* (2004) for frozen storage of green beans.

The effect of blanching temperature and time on ascorbic acid of peas is presented in Table 2. For the blanching temperature, the maximum ascorbic acid of peas (28.74 mg/100g) was obtained for 70 °C and minimum ascorbic acid of peas (26.24 mg/100g) was obtained for 100 °C at end of 180 days of storage period. The minimum percentage decrease (8.37%) was found for the 90 °C temperature and the maximum percentage decrease (10.23%) was found for the 100 °C temperature. The statistical analysis of the data showed that the effect of blanching temperature on the ascorbic acid of peas was found significant at the end of storage period at 5% level of significance.

Effect of different blanching times showed that the maximum ascorbic acid of peas (28.83 mg/100g) was obtained for the blanching time of 1 min and

minimum (26.89 mg/100g) was obtained 3 min of blanching time at the end of 180 days of storage period. The minimum percentage decrease in ascorbic acid of peas (8.19%) was found when the blanching time was 3 min and the maximum percentage decrease in ascorbic acid of peas (10.72%) was found for the blanching time of 1 min. The statistical analysis of blanching time showed that the ascorbic acid of peas was significant at the end of storage period at 5% level of significance. The interaction effect of blanching temperature and blanching time on ascorbic acid of peas was significant at the end of storage period at 5% level of significance (Table 2).

### Effect of blanching temperature and time on overall acceptability of peas

The treatment wise effect of blanching temperature and time on overall acceptability of peas is shown graphically in Fig. 6. It is evident from the figure that overall acceptability of peas decreased with increase in storage period in all treatments. The maximum score of overall acceptability of peas (6.45) was found for the treatment  $T_5$  (80 °C temperature and 2 min) after 180 days of storage period (Table 1). The minimum score of overall acceptability of peas (4.00) was found for the treatment  $T_2$  (70 °C temperature and 2 min) at end of 180 days of storage period. The minimum percentage decrease (15.16%) was found in treatment  $T_5$  (80 °C temperature and 2 min) and the maximum percentage decrease (46.64%) was found in treatment  $T_2$  (70 °C temperature and 2 min).



The effect of blanching temperature and time on overall acceptability of peas is presented in Table 2. For the blanching temperature, the maximum score of overall acceptability of peas (6.03) was obtained for 80 °C and minimum score of overall acceptability of peas (4.73) was obtained for 70 °C at end of 180 days of storage period. The minimum percentage decrease (19.28%) was found for the temperature T2 (80 °C temperature) and the maximum percentage decrease (36.34%) was found for the temperature T1 (70 °C temperature). The statistical analysis of the data showed that the effect of blanching temperature on the overall acceptability of peas was found significant at the end of storage period at 5% level of significance.

Effect of different blanching times showed that the maximum score of overall acceptability of peas (5.68) was obtained for the blanching time of 3 min and minimum (5.18) was obtained 1 min of blanching time at the end of 180 days of storage period. The minimum percentage decrease in overall acceptability of peas (23.24%) was found when the blanching time was 3 min and the maximum percentage decrease in overall acceptability of peas (30.66%) was found for the blanching time of 1 min. The statistical analysis of blanching time showed that the overall acceptability of peas was found significant at the end of storage period at 5% level of significance. The interaction effect of blanching temperature and blanching time on overall acceptability of peas was found significant at the end of storage period at 5% level of significance (Table 2).

## CONCLUSION

The maximum firmness of peas (416.60 g) was found in the treatment T<sub>5</sub> (80 °C temperature and 2 min) after 180 days of storage period. The maximum protein content of peas (5.87 %) and carbohydrate (14.79 %) was found in the treatment T<sub>14</sub> (unblanched) after 180 days of storage period. The maximum ascorbic acid (29.92 mg/100g) and chlorophyll (1.11 mg/g) was found in the treatment T<sub>5</sub> (80 °C temperature and 2 min) after 180 days of storage period. The maximum score of overall acceptability (6.45) was found a in the treatment T<sub>5</sub> (80 °C temperature and 2 min) after 180 days of storage period. The minimum changes of firmness (465.75 to 416.60 g), ascorbic acid (31.49 to 29.92

mg/100g), chlorophyll (1.20 to 1.11 mg/g) and sensory score (7.60 to 6.45) was observed in 80 °C temperature and 2 min blanched in 4% maltose solution. Considering the overall aspects of the study, it may be concluded that treatment T<sub>5</sub> (80 °C temperature and 2 min blanched in 4% maltose solution) was observed to be best treatment amongst all treatments considering firmness, ascorbic acid, chlorophyll and sensory characteristics of green pea at the end of storage period.

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# Physical Properties of Fresh Turmeric Rhizomes (Var. Salem)

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## ABSTRACT

The sample of 50 turmeric rhizomes (var. salem) was selected for analysing their physical properties. The average weight of mother and finger rhizome were found 54.48 g and 28.15 g, respectively. The average values of their geometric properties viz. length (major dimension) ( $65.093 \pm 9.11$  mm), width (intermediate dimension) ( $40.58 \pm 5.56$  mm), thickness (minor dimension) ( $38.34 \pm 3.24$  mm), arithmetic mean diameter ( $51.36 \pm 4.76$  mm), square mean diameter ( $86.41 \pm 7.80$  mm), equivalent diameter ( $62.18 \pm 5.63$  mm), aspect ratio ( $0.54 \pm 0.07$ ), surface area ( $6341.39 \pm 1106.62$  mm<sup>2</sup>), unit volume ( $42162.05 \pm 10765.23$  mm<sup>3</sup>), shape factor ( $1.03 \pm 0.07$ ) were reported for mother rhizomes. The average size and sphericity for mother rhizomes were found  $48.77 \pm 4.35$  mm and  $0.652 \pm 0.046$ , respectively. The mean value of the gravimetric properties viz. bulk density, true density and porosity of mother rhizome were observed  $570.38 \pm 46.83$  kg/m<sup>3</sup>,  $1083.20 \pm 88.72$  kg/m<sup>3</sup> and  $47.33 \pm 1.04\%$ , respectively. The average values of their geometric properties viz. length ( $61.02 \pm 19.51$  mm), width ( $21.28 \pm 3.43$  mm), thickness ( $19.59 \pm 3.52$  mm), arithmetic mean diameter ( $33.95 \pm 8.00$  mm), square mean diameter ( $53.62 \pm 11.08$  mm), equivalent diameter ( $38.92 \pm 8.20$  mm), aspect ratio ( $0.375 \pm 0.10$ ), surface area ( $2422.52 \pm 972.91$  mm<sup>2</sup>), unit volume ( $8691.42 \pm 4777.80$  mm<sup>3</sup>), shape factor ( $1.050 \pm 0.062$ ) were reported for finger rhizomes. The average size and sphericity for finger rhizomes were found  $29.17 \pm 5.65$  mm and  $0.501 \pm 0.090$ , respectively. The mean value of the gravimetric properties viz. bulk density, true density and porosity of finger rhizomes were observed  $492.11 \pm 52.98$  kg/m<sup>3</sup>,  $1251.17 \pm 59.67$  kg/m<sup>3</sup> and  $63.96 \pm 2.54\%$ , respectively.

## HIGHLIGHTS

- The article focuses on the physical properties of mother and finger rhizomes.
- The arithmetic mean diameter, geometric mean diameter, square mean diameter and equivalent diameter showed an increasing trend with an increase in length, width and thickness of rhizomes.
- The sphericity, aspect ratio and shape factor of turmeric rhizomes were found to be decreased with an increase in lateral dimension of rhizomes.

**Keywords:** Turmeric rhizome, Finger rhizome, Mother rhizome, physical properties, variety salem, geometric properties, gravimetric properties

India has been renowned as the “Spice Bowl of World” since it has cultivated a vast range of high-quality spices. India is the world’s greatest producer, buyer and exporter of spices. Turmeric is known as “Indian saffron”, is an ancient spice derived from the rhizomes of *Curcuma longa*, a ginger family (Zingiberaceae) member and is often referred to as “Haldi” or “Haridra”. The crop may be sown in April-May with pre-monsoon rains and harvested within 7-9 months of sowing in Kerala and other

West Coast places when rainfall comes early. Turmeric rhizome yields range from 20,000 to 35,000 kg/ha, depending on variety. It is one of the most important export crops for India as it contributes to around 80 % of total global production. Turmeric accounts for around 6% of the entire area under

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spice and condiment cultivation in India and it plays a significant role in the Indian economy (Choudhary and Rahi 2018). Telangana, Karnataka, Tamil Nadu, Andhra Pradesh and West Bengal are the top turmeric-producing states in India. Turmeric is grown in India in roughly 70 cultivars or variations, with some major regional trade varieties including 'Rajapuri', 'Duggirala', 'Cuddappah', 'Berhampur', 'Erode', 'Nizamabad', 'Koraput', 'Kasturi', 'Chaya', 'Kodur', 'Salem', 'Waigon', 'Alleppey', 'Karur', 'Tekurpeta' (Sasikumar 2005).

Turmeric is a perennial plant having 600-900 mm height with a short stem, large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow in colour. Turmeric has primary and secondary rhizomes that can be present in different from spherical to slightly conical hemispherical and cylindrical (Balsubramanian *et al.* 2012). The primary rhizomes are called the "mother rhizomes" or bulb, which is pear-shaped in the centre. The branches of mother rhizomes are the secondary rhizomes, called lateral or "finger rhizomes" (Hossain 2010).

Food products like turmeric are often characterized based on their physical properties since these properties are utilized for sorting and grading (de Ramos *et al.* 2021). The study of turmeric physical properties aids in the design of various agricultural machines used in its processing chain like the washers, graders, boilers, driers, polishers and storage devices. Its comprehension provides a better grasp of the many physical processes of harvesting, transportation from the field to the processing unit and shipping within the processing unit (Boudh and Riopel 2015). If the handling system is not efficiently designed it can lead to excessive cost. Hence its calculation leads to the determination of various dimensions of the machines.

Review of literature revealed that the lack of studies on the physical properties of fresh salem turmeric rhizomes. The dimensions, weight, bulk density, true density and the projected area of agricultural product changes with a variety of product, agronomical conditions that product was grown and moisture content of product (Konak *et al.* 2002). Physical characteristics are primarily determined by the genetic constitution of the cultivars. But these are also influenced by agro climatic factors and farming practices prevalent

in various regions. Hence, an attempt was made to study various of geometric properties viz. length, width, thickness, geometric mean diameter, arithmetic mean diameter, Square mean diameter, Equivalent diameter, aspect ratio, Unit volume, Surface area, sphericity and shape factor and gravimetric properties viz. Bulk density, true density and porosity of raw fresh turmeric rhizomes.

## MATERIALS AND METHODS

### Sample preparation

The most popular turmeric cultivar salem was purchased from the farmer of sultanpur, district Rajkot for the given experiment. The fresh turmeric rhizomes were washed thoroughly in tap water to remove the adhering soil, hairs and extraneous matter. The undesirable portions were removed manually and then the rhizomes were again washed and cleaned properly. This washed and cleaned fresh turmeric rhizomes were used for further study.

### Moisture content of fresh turmeric rhizomes

Estimation of moisture is necessary prior to the assessment of physical properties as they vary with moisture content. So, moisture content of turmeric rhizomes was determined before measuring the physical properties. The moisture content (% wb) of fresh turmeric rhizomes was determined by hot air oven method as described by AOAC (2005). The moisture content was calculated using the following formula:

$$\text{Moisture content (\% wb)} = \frac{W_i - W_o}{W_i}$$

### Measurement of physical properties of finger rhizomes and mother rhizomes

#### Geometric properties

The sample of 50 turmeric rhizomes was randomly selected for analysing their physical properties. The dimensions of length (major dimension); L, width (intermediate dimension); W and thickness (minor dimension); T in millimetres were measured using a digital Vernier calliper having an accuracy of 0.01 mm. The average geometric mean diameter (GMD) or size, sphericity, arithmetic mean diameter (AMD),



square mean diameter (SMD), equivalent diameter (ED), aspect ratio (AR) were determined by using the following equations.

### Size or geometric mean diameter (GMD)

The geometric mean diameter (GMD) was considered as the size criterion and was expressed as the cube root of three axes of rhizome using the major dimension (L), intermittent (W) and minor dimension (T). The size was calculated using the following formula given by Mohsenin (1986):

$$\text{Size (mm)} = (L \times W \times T)^{1/3}$$

### Sphericity

The sphericity were calculated using the following formula given by Mohsenin (1986):

$$\text{Sphericity} = \frac{(L \times W \times T)^{1/3}}{L}$$

### Arithmetic mean diameter (AMD)

The Arithmetic mean diameter is calculated using the following formula given by Mohsenin (1986):

$$\text{Arithmetic mean diameter (mm)} = \frac{L \times W \times T}{3}$$

### Square mean diameter (SMD)

The Square mean diameter was calculated using the following formula given by Mohsenin (1986):

$$\text{Square mean diameter (mm)} = \sqrt{LW + WT + LT}$$

### Equivalent diameter (ED)

The Equivalent diameter was calculated using the following formula given by Mohsenin (1986):

$$\text{Equivalent diameter (mm)} = \frac{GMD + AMD + SMD}{3}$$

### Aspect ratio (AR)

The Aspect ratio was calculated using the following formula as given by Mohsenin (1986):

$$\text{Aspect ratio} = \frac{W}{L}$$

### Unit volume and surface area

The unit volume (V) and surface area (S) were determined as per the following equations (Jain and Bal, 1997):

$$\text{Surface area (mm}^2\text{)} = \frac{\pi BL^2}{2L - B}$$

$$\text{Unit Volume (mm}^3\text{)} = \frac{\pi B^2 L^2}{6(2L - B)}$$

where,  $B = \sqrt{WT}$

### Shape Factor

Shape factor based on the unit volume and surface area was determined (Balasubramanian *et al.* 2012) as:

$$\text{Shape factor} = D/C$$

Where,  $C = V/W$

$$D = S / 6W^2$$

### Gravimetric properties

The gravimetric properties like thousand seed weight, bulk density, true density and porosity were measured and replicated thrice.

### Thousand rhizome weight

Thousand rhizome weight was taken manually by counting a thousand rhizomes and weighing the rhizomes in an electronic balance with an accuracy of 0.001 g.

### Bulk density

The bulk density was measured by taking the mass of the sample filled in a square box of dimension 21 cm × 21 cm × 19 cm from a constant height. The bulk density (kg/m<sup>3</sup>) was calculated using the following formula (Mohsenin 1986):

Bulk density (kg/m<sup>3</sup>) =

$$\frac{\text{Weight of sample (g)}}{\text{Total volume occupied by sample (cm}^3\text{)}} \times 1000$$



### True density

True density of the turmeric rhizomes was determined by the toluene displacement method. Weight of single turmeric rhizome and that of beaker plus toluene was taken as  $W_1$  and  $W_2$  respectively. Then turmeric rhizome was submerged in the beaker without touching the side of beaker and bottom of the beaker and the weight was taken ( $W_3$ ). Then the weight of toluene displaced ( $W_4 = W_3 - W_2$ ) was measured. (Mohsenin 1986; Sacilik *et al.* 2003):

Volume of toluene displaced (V) =

$$\frac{W_4 (g)}{\text{Specific gravity of toluene (g/cm}^3\text{)}}$$

$$\text{True density (kg/m}^3\text{)} = \frac{W_1 (g)}{V (cm^3)} \times 1000$$

### Porosity

The porosity was calculated using from the values of true and bulk density using the relationship given by Mohsenin (1986):

$$\text{Porosity (\%)} = \left( 1 - \frac{\text{Bulk density}}{\text{True density}} \right) \times 100$$

## RESULTS AND DISCUSSION

### Physical Properties of mother rhizomes

#### Geometric properties

The moisture content of raw turmeric rhizomes was  $84.23 \pm 0.64$  (% w.b.). The length, width and thickness of turmeric rhizomes was varied from 49.360 to 81.22, 33.54 to 51.45 and 32.60 to 43.98 mm, respectively. The mean of length, width and thickness was estimated  $65.093 \pm 9.11$ ,  $40.58 \pm 5.56$  and  $38.34 \pm 3.24$  mm for the turmeric rhizomes. The mean weight of mother rhizomes was 54.48 g. The size of rhizomes was varied from 41.93 to 55.34 mm with a mean value of  $48.77 \pm 4.35$  mm. The sphericity of mother rhizomes was varied from 0.574 to 0.726 with a mean value of  $0.652 \pm 0.046$ . The arithmetic mean diameter, square mean diameter and equivalent were varied from 43.63

to 58.42, 73.99 to 98.12 and 53.22 to 70.63 mm, respectively with a mean value of  $51.36 \pm 4.76$ ,  $86.41 \pm 7.80$  and  $62.18 \pm 5.63$  mm, respectively. The mean value of aspect ratio was estimated  $0.54 \pm 0.07$ . The surface area and unit volume of fresh rhizomes was varied from 4650.40 to 8083.02 mm<sup>2</sup> and 26651.39 to 59490.51 mm<sup>3</sup>. The mean value of surface area and unit volume was  $6341.39 \pm 1106.62$  mm<sup>2</sup> and  $42162.05 \pm 10765.23$  mm<sup>3</sup> with a coefficient of variation was 17.45 and 25.53 %, respectively. The range of shape factor was 0.92 to 1.16 with a mean value of  $1.03 \pm 0.07$  and value of the geometric properties shows in Table 1.

### Gravimetric properties

The thousand rhizome weight was varied from 61.79 to 57.21 kg. The mean value of the thousand rhizome weight was 59.48 with a coefficient variation of 2.82%. The bulk density and true density were varied from 522.019 to 627.28 and 981.26 to 1202.58 kg/m<sup>3</sup> with a mean value of  $570.38 \pm 46.83$  and  $1083.20 \pm 88.72$  kg/m<sup>3</sup>, respectively. The coefficient of variation for bulk density and true density was estimated 8.21 and 8.19 %, respectively. The value of porosity was ranged from 46.06 to 48.80%. The mean value of porosity was estimated  $47.33 \pm 1.04$  and coefficient of variation was estimated 2.19 %. Khambalkar *et al.* (2017) reported that the porosity of mother rhizomes varied from 32 to 63%. They reported average bulk density, true density and porosity of mother rhizomes 529.66 kg/m<sup>3</sup>, 1018.95 kg/m<sup>3</sup> and 46% respectively and value of all the gravimetric properties represented in Table 2.

### Physical Properties of finger rhizomes

#### Geometric properties

The turmeric finger rhizomes have branching of varying size and shape. Hence, relatively smaller primary and secondary development (fingers) were removed and all the measurements were made only for main fingers as reported by Dhananchezhayan *et al.* (2020). Table 3 shows the maximum value, minimum value, mean values, standard deviation and coefficient of variation of all geometric properties of the fresh turmeric rhizomes. The length, width and thickness of turmeric rhizomes was varied from 29.21 to 103.29, 14.15 to 31.07 and 10.40 to 28.70 mm, respectively. The mean of length, width

**Table 1:** Geometric properties of mother rhizomes

Sl. No.	Parameter	Maximum	Minimum	Mean	SD	C.V. %
1	Length (L), mm	81.22	49.360	65.093	9.11	12.11
2	Width (W), mm	51.45	33.54	40.58	5.56	13.71
3	Thickness (T), mm	43.98	32.60	38.34	3.24	8.46
4	Weight (w), g	84.66	26.69	54.48	17.75	32.58
5	Size (GMD), mm	55.34	41.93	48.77	4.35	8.92
6	Sphericity	0.726	0.574	0.652	0.046	7.020
7	Arithmetic mean diameter (AMD), mm	58.42	43.63	51.36	4.76	9.27
8	Square mean diameter (SMD), mm	98.12	73.99	86.41	7.80	9.03
9	Equivalent diameter (ED), mm	70.63	53.22	62.18	5.63	9.03
10	Aspect ratio (AR)	0.71	0.46	0.54	0.07	13.59
11	Surface Area (S), mm <sup>2</sup>	8083.02	4650.40	6341.39	1106.62	17.45
12	Unit volume (V), mm <sup>3</sup>	59490.51	26651.39	42162.05	10765.23	25.53
13	Shape Factor	1.16	0.92	1.03	0.07	7.08

**Table 2:** Gravimetric properties of mother rhizomes

Sl. No.	Parameter	Maximum	Minimum	Mean	SD	C.V. %
1	Thousand rhizome weight, kg	61.79	57.21	59.48	1.68	2.82
2	Bulk density, kg/m <sup>3</sup>	627.28	522.01	570.38	46.83	8.21
3	True density, kg/m <sup>3</sup>	1202.58	981.26	1083.2	88.72	8.19
4	Porosity, %	48.800	46.069	47.33	1.04	2.19

**Table 3:** Geometric properties of finger rhizomes

Sl. No.	Parameter	Maximum	Minimum	Mean	SD	C.V. %
1	Length (L), mm	103.290	29.210	61.020	19.510	31.970
2	Width (W), mm	31.070	14.150	21.280	3.430	16.110
3	Thickness (T), mm	28.700	10.400	19.590	3.520	17.950
4	Weight (w), g	42.030	11.260	28.150	7.660	27.220
5	Size (GMD), mm	41.681	18.722	29.170	5.650	19.370
6	Sphericity	0.708	0.355	0.501	0.090	17.960
7	Arithmetic mean diameter (AMD), mm	51.150	21.343	33.960	8.000	23.570
8	Square mean diameter (SMD), mm	76.408	34.336	53.620	11.080	20.660
9	Equivalent diameter (ED), mm	55.852	24.874	38.920	8.200	21.080
10	Aspect ratio (AR)	0.614	0.212	0.375	0.100	26.630
11	Surface area (S), mm <sup>2</sup>	4718.25	950.75	2422.520	972.910	40.160
12	Unit volume (V), mm <sup>3</sup>	22784.173	2069.124	8691.420	4777.800	54.970
13	Shape Factor	1.416	0.981	1.050	0.062	5.960

and thickness was estimated  $61.02 \pm 19.51$ ,  $21.28 \pm 3.43$  and  $19.59 \pm 3.52$  mm for the turmeric rhizomes. Balasubramanian *et al.* (2012) also determined physical properties of turmeric and they recorded a length, width and thickness ranging from 30.38 – 50.60, 9.77 – 10.64, and 5.18 – 6.44 mm, respectively, which are relatively lower. This variation is due to different variety and locality of the sample. The weight of fresh single piece turmeric rhizome was varied from 11.26 to 42.03 g. The mean of weight was 28.15 g with a standard deviation of 27.22 % for fresh turmeric rhizomes. The size of rhizomes was varied from 18.72 to 41.68 mm with a mean value

of  $29.17 \pm 5.65$  mm. The shape of turmeric rhizomes could be expressed in terms of their sphericity. The sphericity of turmeric rhizomes was varied from 0.355 to 0.708 with a mean value of  $0.501 \pm 0.090$ . Similar, result found by de Ramos *et al.* (2021) for sphericity. They reported that sphericity varied from 0.289-0.755 for fresh finger rhizomes. The arithmetic mean diameter, square mean diameter and equivalent were varied from 21.34 to 51.15, 34.336 to 76.408 and 24.87 to 55.85 mm, respectively with a mean value of  $33.95 \pm 8.00$ ,  $53.62 \pm 11.08$  and  $38.92 \pm 8.20$  mm, respectively. The surface area and unit volume of fresh rhizomes was varied from



950.75 to 4718.25 mm<sup>2</sup> and 2069.12 to 22784.17 mm<sup>3</sup>. The mean value of surface area and unit volume was 2422.52 ± 972.91 mm<sup>2</sup> and 8691.42 ± 4777.80 mm<sup>3</sup> with a coefficient of variation was 40.16 and 54.97%, respectively. From these values, the volume of the turmeric yielded the highest coefficient of variation with 54.97 % while the geometric mean diameter yielded the lowest with 19.37 %. The mean value of aspect ratio was estimated 0.375 ± 0.10. The range of shape factor was 0.981 to 1.416 with a mean value of 1.050 ± 0.062 with a CV of only 5.96%.

The dimensions of turmeric rhizomes are not uniform. So, number of measurement data would be needed to describe them accurately. In all the three axial dimension length, width and thickness, the high variation is observed in the length than that of width and thickness (Fig. 1). It is observed that the variation in geometric properties mainly due to variation in length. The arithmetic mean diameter, geometric mean diameter, square mean diameter and equivalent diameter showed an increasing trend with the increase in length, width and thickness of rhizomes.

The variation in size of fresh rhizomes with respect to length, width and thickness is shown in Fig. 2, 3 and 4. The highest variation was observed with length having R<sup>2</sup> value of 0.7785 and lowest variation

was observed with thickness having R<sup>2</sup> value of 0.7489. The best-suited equation for variation in size with length was observed as,  $y = 0.4181 \times 1.4704$  suggesting the power of 1.4704 variation in size with length. Similarly, the best-suited equation for variation in size with width was observed as,  $y = 1.8976 \times 0.7168$  with the highest R<sup>2</sup> value as 0.7509 suggesting the power of 0.7168 variation in size with width. The relationship between size and thickness was fitted with the linear equation  $y = 0.5385x + 3.8806$  with the highest R<sup>2</sup> value of 0.7489.

The variation in equivalent diameter of fresh rhizomes with respect to length, width and thickness is shown in Fig. 5, 6 and 7. The best-suited equation for variation in equivalent diameter with length was observed as,  $y = 0.0089x^2 + 1.5075x - 11.772$  with the highest R<sup>2</sup> value as 0.8767 suggesting the polynomial variation with order 2 in equatorial diameter with weight. The best-suited equation for variation in equivalent diameter with width was observed as,  $y = 2.2146 \times 0.6183$  with the highest R<sup>2</sup> value as 0.6599 suggesting the power of 0.6183 variation. The variation in equivalent diameter with thickness was also found linear with the best suited equation as  $y = 0.3418x + 6.285$  with the highest R<sup>2</sup> value of 0.6359.

The sphericity, aspect ratio and shape factor

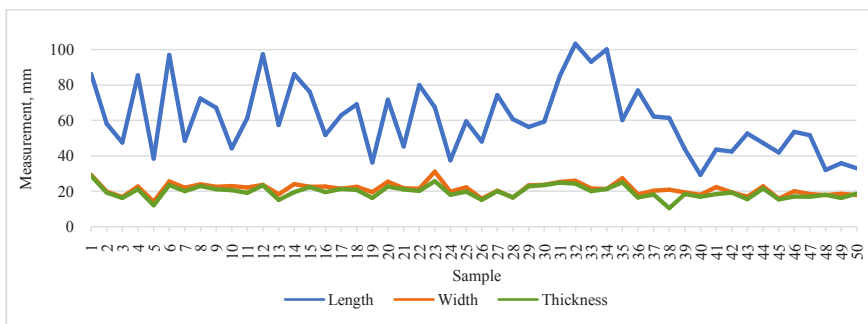


Fig. 1: Variation in dimensions

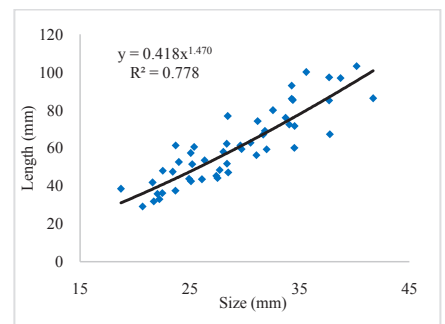


Fig. 2: Variation in size with length

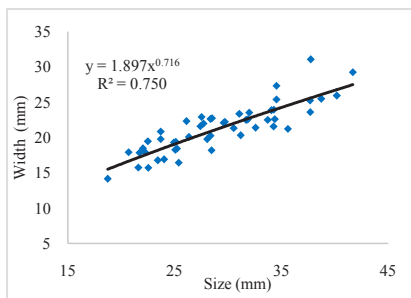


Fig. 3: Variation in size with width

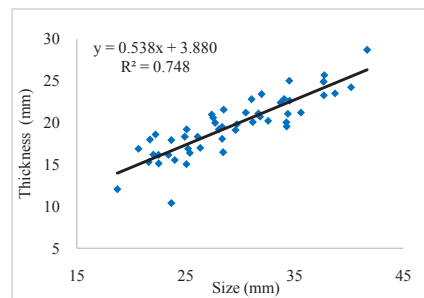


Fig. 4: Variation in size with thickness

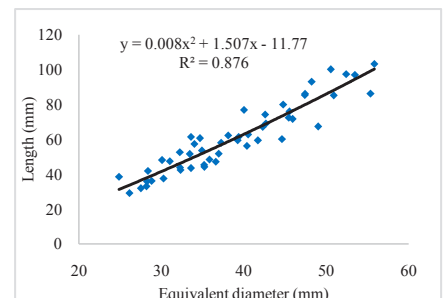


Fig. 5: Variation in ED with length

of turmeric rhizomes decreased with increase in dimension. This may be attributed to the irregular shape of turmeric rhizomes. The variation in sphericity with length was also found the exponential with the best suited equation  $y = 273.52e - 3.093x$  with the highest  $R^2$  value of 0.7371. (Fig. 8). The variation in sphericity is mainly due to variation in length. While the variation in width and thickness has small or low effect in variation in sphericity. However, the variation in sphericity with width and thickness was found non-significant (Fig. 9 and 10).

The variation in weight of fresh rhizomes with respect to size and equivalent diameter is shown in Fig. 11 and 12 respectively. The best-suited equation for variation in weight with was observed as,  $y =$

$-0.0365x^2 + 3.4639x - 40.633$  with the highest  $R^2$  value as 0.9666 suggesting the polynomial variation with order 2 in size with weight. Similarly, the variation in weight with equivalent diameter was also found the polynomial variation with order 2 with the best suited equation  $y = -0.0187x^2 + 2.3874x - 35.047$  with the highest  $R^2$  value of 0.9687. The variation in weight of fresh rhizomes with respect to surface area and unit volume is shown in Fig. 13 and 14, respectively. The best-suited equation for variation in weight with was observed as,  $y = 18.155\ln(x) - 111.8$  with the highest  $R^2$  value as 0.9687 suggesting the logarithmic variation in surface area with weight. Similarly, the variation in weight with unit volume was also found the logarithmic with the best suited equation  $y = 13.05\ln(x) - 88.214$  with the highest  $R^2$  value of 0.9407.

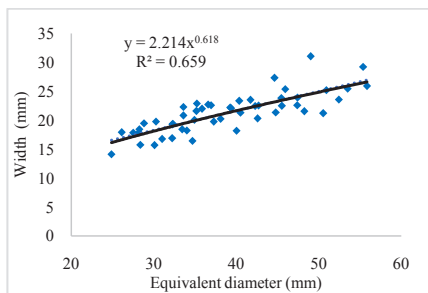


Fig. 6: Variation in ED with width

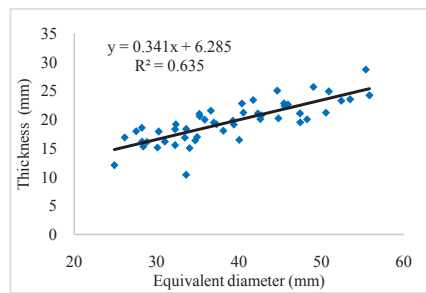


Fig. 7: Variation in ED with thickness

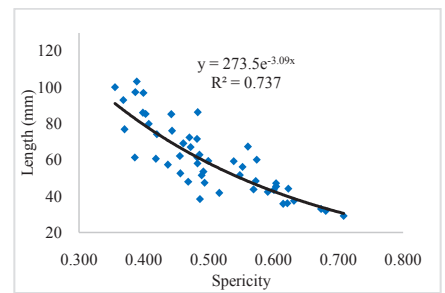


Fig. 8: Variation in sphericity with length

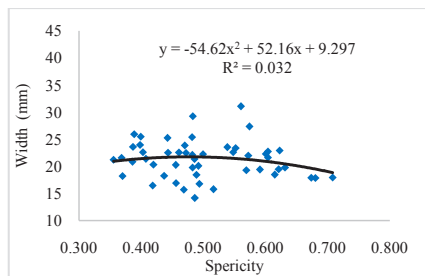


Fig. 9: Variation in sphericity with width

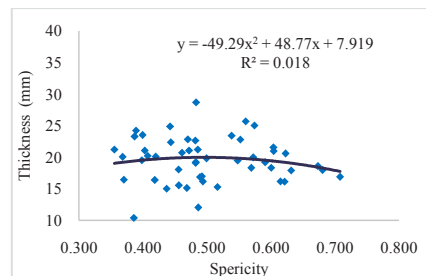


Fig. 10: Variation in sphericity with thickness

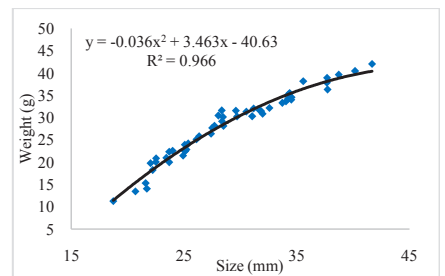


Fig. 11: Variation in weight with size

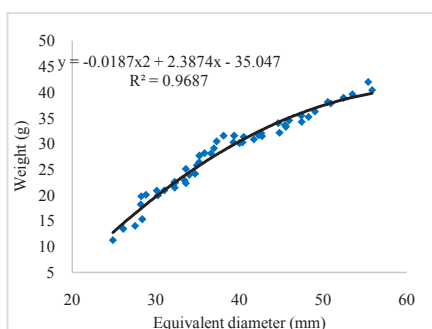


Fig. 12: Variation in weight with ED

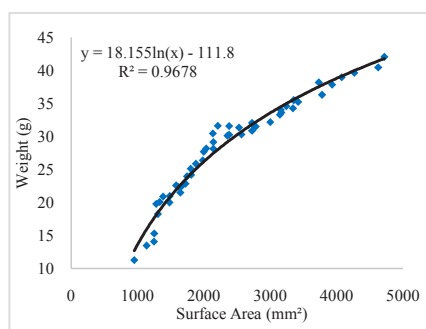


Fig. 13: Variation in weight with surface area

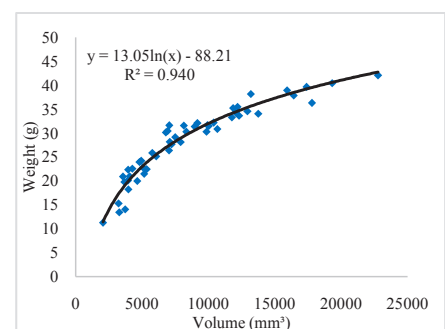


Fig. 14: Variation in weight with volume



## Gravimetric properties

Table 4 shows the maximum value, minimum value, mean values, standard deviation and coefficient of variation of 1000 rhizome Weight, Bulk Density, True Density and porosity. The thousand rhizome weight was varied from 27.45 to 32.29 kg. The mean value of the thousand rhizome weight was  $29.72 \pm 1.9$  with a coefficient variation of 6.4%. The bulk density was varied from 422.09 to 527.33 with a mean value of  $492.11 \pm 52.98$ . De Ramos *et al.* 2021 reported that the bulk density varied from 0.489 to 0.519 g/cm<sup>3</sup>. True density was varied from 1186.78 to 1334.77 kg/m<sup>3</sup> with a mean value of and  $1251.17 \pm 59.67$  kg/m<sup>3</sup>. The coefficient of variation for bulk density and true density was estimated 11.72 and 4.77 %, respectively. The value of porosity was ranged from 60.49 to 67.48%. The mean value of porosity was estimated  $63.96 \pm 2.54$  and coefficient of variation was estimated 3.97 %. Balakrishnan *et al.* (2020) also found  $60.15 \pm 2.31\%$  porosity for fresh finger rhizomes.

**Table 4:** Gravimetric properties of finger rhizomes

Sl. No.	Parameter	Max.	Mini.	Mean	SD	C.V. %
1	Thousand rhizome Weight, kg	32.29	27.45	29.72	1.9	6.4
2	Bulk density, kg/m <sup>3</sup>	527.33	422.09	492.11	52.98	11.72
3	True density, kg/m <sup>3</sup>	1334.77	1186.78	1251.17	59.67	4.77
4	Porosity, %	67.48	60.49	63.96	2.54	3.97

## CONCLUSION

This work focuses on physical properties of salem cultivar of the turmeric. Different physical properties including geometric and gravimetric properties of mother and finger rhizomes were measured and given in results. Generally, finger rhizomes are more useful as compared to mother rhizome, therefore work focuses on properties of finger rhizomes. The size and sphericity of finger rhizomes were varied from 18.72 to 41.68 and 0.355 to 0.708 mm, respectively. The arithmetic mean diameter, square mean diameter and equivalent were varied from 21.34 to 51.15, 34.336 to 76.408 and 24.87 to 55.85 mm, respectively. The surface area and unit volume of finger rhizomes was varied

from 950.75 to 4718.25 mm<sup>2</sup> and 2069.12 to 22784.17 mm<sup>3</sup>. The range of shape factor was 0.981 to 1.416. The thousand rhizome weight of finger rhizomes was varied from 27.45 to 32.29 kg. The bulk density and true density were varied from 385.96 to 527.33 and 1186.78 to 1334.77 kg/m<sup>3</sup>, respectively. The value of porosity was ranged from 60.49 to 67.48%. The size of mother rhizomes was varied from 41.93 to 55.34 mm with a mean value of  $48.77 \pm 4.35$  mm. The sphericity of mother rhizomes was varied from 0.574 to 0.726 with a mean value of  $0.652 \pm 0.046$ . The arithmetic mean diameter, square mean diameter and equivalent were varied from 43.63 to 58.42, 73.99 to 98.12 and 53.22 to 70.63 mm, respectively. The surface area and unit volume of fresh rhizomes was varied from 4650.40 to 8083.02 mm<sup>2</sup> and 26651.39 to 59490.51 mm<sup>3</sup>. The range of shape factor for mother rhizomes was 0.92 to 1.16.

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# Development of Palm Sugar Substituted Yoghurt

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## ABSTRACT

A detailed study was carried out to substitute cane sugar with palm sugar in the preparation of yoghurt. The yoghurt samples were prepared by replacing cane sugar with palm sugar at 25%, 50%, 75% and 100% levels, and were designated as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively. The yoghurt sample without replacement by palm sugar was taken as control. The physicochemical, textural, microbial and sensory properties of the control and treatment yoghurt samples were evaluated. There found to be no variation in the curd setting time of control and treatment samples. All the yoghurt samples uniformly maintained the pH range of 4.54 to 4.57. The fat and total solids content of the control and treatments exhibited no significant difference among them. The textural properties like firmness and consistency of control and treatments exhibited a significant difference (P<0.01). As the cane sugar replacement level increased, the firmness and consistency scores of the treatments also increased. The coliforms and yeast and mould count of control and treatments exhibited no significant difference among them and were within the FSSAI (2012) prescribed limits. A panel of six trained judges carried out the sensory evaluation of the yoghurt samples, and the sensory parameters (such as appearance, body and texture, flavour and overall acceptability) of control and treatments revealed no significant differences among them. Nevertheless, the scores exhibited an increasing trend towards the 100 per cent substitution of cane sugar with palm sugar. Therefore, the substitution of cane sugar with palm sugar in the yoghurt improved the acceptability, as per the scores given by the sensory panel. Hence, it can be concluded that the yoghurt samples can be prepared by replacing cane sugar with palm sugar up to 100 per cent level without affecting the physicochemical, textural, microbial and sensory properties.

## HIGHLIGHTS

- ① Palm sugar replacement did not affect the pH, curd-setting time, fat and total solids content.
- ① Positive correlation was observed between palm sugar replacement level and textural properties like firmness and consistency.
- ① Sensory scores exhibited an increasing trend towards the 100 per cent substitution of cane sugar with palm sugar.

**Keywords:** Yoghurt, Palm sugar, Sensory properties

Among the fermented milk products, yoghurt occupies a major part. Yoghurt is prepared by fermentation of milk by *Streptococcus thermophiles* and *Lactobacillus bulgaricus* (FAO/WHO 1977). Yoghurt is popular among consumers because of its rich taste and creamy, the reality is that its benefits go beyond just its taste. Consumption of fermented dairy products has long been well-thought-out to be beneficial to digestive and overall health

(Morelli 2014). Routine consumption of yoghurt was found to enhance the number of gut beneficial bacteria. Moreover, yoghurt supplies good quality proteins, calcium, phosphorus and potassium, and

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contains significant quantities of general vitamins. In addition to probiotics, yoghurt is a good source of protein, calcium, iodine and vitamin B<sub>12</sub>, and its consumption has been associated with a lower risk of obesity and cardio-metabolic in both children and adults (Marette and Picard-Deland 2014). Probably, the cane sugar aggravates blood sugar in people with diabetes mellitus. For this reason, instead of cane sugar, palm sugar was used to supplement the sugar content in the yoghurt. The palm sugar prepared from *Borassus flabellifer* Linn. (Palmyra palm) exhibits the highest level of antioxidant activity compared to various types of cane sugars, having an antioxidant activity equivalent to 1.7 mg of vitamin C per 1 g of sugar (Sia *et al.* 2010). The major carbohydrate found in the analyzed palm tree syrup samples was sucrose (37.8%) followed by glucose (9.50%) and fructose (4.80%) (Luis *et al.* 2012). Palm sugar powder contains glucose 4.46 (g/100g DW), fructose 4.41 (g/100g DW), sucrose 68.76 (g/100g DW) and total sugars 77.63 (g/100g DW) and similarly, the refined cane sugars contained 0.23, 2.06, 96.42 and 98.71 respectively (Srikaeo *et al.* 2019). Because of their antioxidant properties, there has been a growing interest in the value of polyphenols in palm sugars and other naturally processed sugars, such as non-centrifugal sugar (NCS). Scientific research has confirmed significant positive health effects of NCS and its precursor products, including their immunological effects, anti-toxicity and cytoprotective effects, anti-cariogenic effects, and diabetes and hypertension effects (Vhangani and Van Wyk 2013). Presently, palm sugar is now gaining popularity globally because of its natural source, minimal processing, and healthiness. One of the major health claims of palm sugar is its low glycaemic index (Srikaeo *et al.* 2019). Traditionally, in India, palm sugar is used in ayurvedic traditional medicine preparations. By considering its beneficial properties, palm sugar can be effectively used to replace cane sugar in yoghurt preparation.

The objective of our study was to develop palm sugar substituted yoghurt and to evaluate the physico-chemical, textural, microbial and sensory properties of the developed product.

## MATERIALS AND METHODS

The facilities available at the Department of Livestock product technology (Dairy Science), Veterinary College and Research Institute, Namakkal have been utilized for the preparation of yoghurt samples. Fresh-pooled cow milk was purchased from Livestock Farm Complex, Veterinary College and Research Institute, Namakkal. Spray-dried skim milk powder (Aavin Dairy, Erode) was used to adjust the solids-not-fat content in yoghurt. Ampoules of freeze-dried culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were obtained from National Dairy Research Institute, Karnal, Haryana. Cane sugar, purchased from the local market, was used in the experiment. The freshly prepared palm sugar was purchased directly from the local palm sugar manufacturers at Namakkal.

### Experimental design

The various yoghurt samples designated in the experiment are as follows - Control (Plain yoghurt without substitution of cane sugar with palm sugar), T<sub>1</sub> (25% palm sugar substituted yoghurt), T<sub>2</sub> (50% palm sugar substituted yoghurt), T<sub>3</sub> (75% palm sugar substituted yoghurt) and T<sub>4</sub> (100% palm sugar substituted yoghurt).

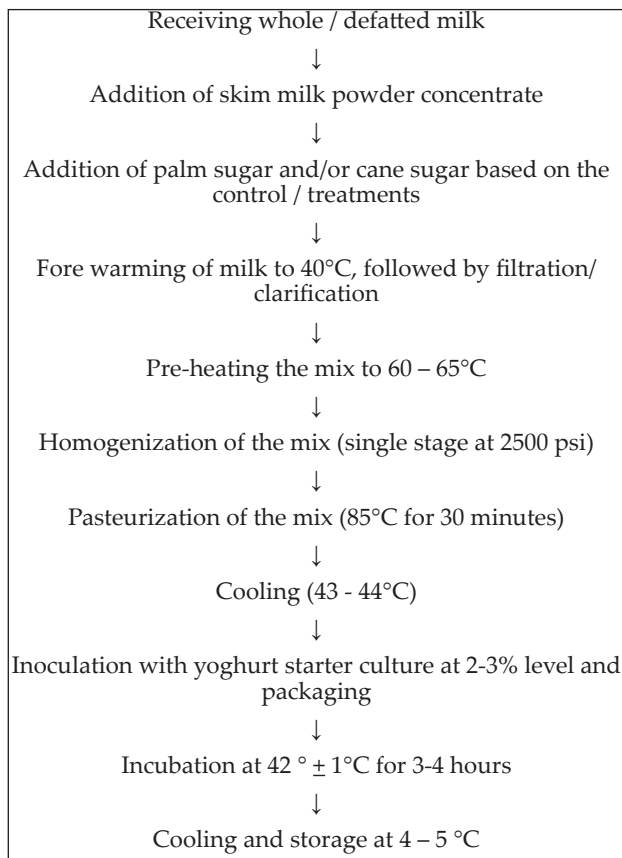
### Preparation of palm sugar substituted yoghurt samples

Yoghurt mixes were prepared by incorporating cow milk, skim milk powder and cane sugar, and the palm sugar was used to replace the cane sugar at 25, 50, 75 and 100 per cent levels. The yoghurt samples were prepared as per the method described by Malarkannan and Geevarghese (1998).

**Table 1:** List of ingredients for the palm sugar substituted yoghurt samples

Ingredients (grams)	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Cow milk	87.5	87.5	87.5	87.5	87.5
Skim milk powder	6.5	6.5	6.5	6.5	6.5
Sugar	6	4.5	3	1.5	—
Palm sugar	—	1.5	3	4.5	6
<b>Total</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>	<b>100.0</b>

## Process flow chart for the preparation of palm sugar substituted yoghurt



### Physicochemical analysis of yoghurt

pH was estimated using a digital pH meter. The total solids content was determined according to AOAC (1990), 15<sup>th</sup> edition. Fat was estimated as per the procedure described in IS:SP:18 (Part XI) – 1981.

### Texture analysis of yoghurt

The Firmness and Consistency of palm sugar substituted yoghurt samples were characterized using the Instron Texture Analyser (Model: TA.XT Plus, Stable Microsystems) and Texture Expert Software. A Back Extrusion Cell (A/BE) with a 35mm disc and extension bar using a 5 kg load cell was used to measure the firmness and consistency scores of the yoghurt samples. Six measurements for each sample were recorded using a 5 mm diameter and 150 mm long stainless steel probe adapter attached to a 5 kg load cell. The penetration depth at the geometrical centre of the samples contained in a standard size back extrusion container (50mm diameter) was 30 mm and the penetration speed was set at 1.0 mm/s. The firmness of the samples was

determined as the peak compression force during penetration. The maximum negative force is taken as the indication of consistency/resistance to flow off the disc during back extrusion. All determinations were carried out at 15°C.

### Sensory evaluation

The yoghurt samples were evaluated for their appearance and colour, flavour, body and texture and overall acceptability by a panel of six judges, using a 9-point Hedonic scale (Dubey *et al.* 2011). All the samples were appropriately coded before being subjected to sensory evaluation by the sensory panel.

### STATISTICAL ANALYSIS

The data obtained in all the experiments were analysed statistically by applying one-way ANOVA by approved statistical methods of SPSS (version 28.0.1.1).

## RESULTS AND DISCUSSION

### Proximate analysis of palm sugar

The proximate analysis of palm sugar revealed 0.4% moisture, 0.87% crude protein, 0.07% crude fibre, 1.63% ether extract, 0.97% total ash and 4048 kcal/kg gross energy.

### Physicochemical analysis of yoghurt

The results of the physicochemical properties of control and treatment yoghurt samples are presented in Table 2.

**Table 2:** Physicochemical properties of yoghurt samples

Parameters	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Setting time (hours)	4.16 ± 0.03	4.15 ± 0.02	4.17 ± 0.01	4.15 ± 0.02	4.18 ± 0.02
pH	4.55 ± 0.02	4.54 ± 0.01	4.56 ± 0.01	4.54 ± 0.01	4.57 ± 0.01
Fat (%)	3.01 ± 0.12	3.02 ± 0.13	3.04 ± 0.09	3.01 ± 0.11	3.06 ± 0.10
Total solids (%)	12.86 ± 0.02	12.85 ± 0.04	12.83 ± 0.02	12.88 ± 0.03	12.89 ± 0.02

There was found to be nil variation in the curd setting time due to the substitution of palm sugar with cane sugar. The results of the control and

treatments are in accordance with the values reported by Malarkannan and Geevarghese (1998) and Ghadge *et al.* (2008). The pH value of control and treatments exhibited no significant difference among them and were within the limits prescribed by FSSAI (2012). The fat and total solids content of the control and treatments exhibited no significant difference among them and were within the FSSAI (2012) prescribed limits of 3.0 and 13.5 per cent respectively.

### Textural and microbial analysis of yoghurt samples

The results pertaining to the textural and microbiological properties of control and treatment yoghurt samples are presented in Table 3. The firmness of control and treatments exhibited a significant difference ( $P < 0.01$ ) among them and exhibited an increasing trend towards 100 per cent replacement of cane sugar with palm sugar. The consistency of control and treatments exhibited a significant difference ( $P < 0.01$ ) among them. As the cane sugar replacement level increased, the consistency scores of the treatments also tend to be increased, which were in accordance with the findings of Yilmaz-Ersan and Topcuoglu (2022).

**Table 3:** Textural and microbiological properties of yoghurt samples

Parameters	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Firmness (g)	107.59 <sup>b</sup> ± 1.55	140.16 <sup>a</sup> ± 3.43	150.88 <sup>a</sup> ± 3.23	155.09 <sup>a</sup> ± 2.81	155.42 <sup>a</sup> ± 2.91
Consistency (g-sec)	2012.73 <sup>e</sup> ± 20.29	2184.33 <sup>d</sup> ± 54.13	2586.94 <sup>c</sup> ± 56.39	2889.26 <sup>b</sup> ± 29.58	3103.91 <sup>a</sup> ± 43.15
Coliform count (cfu/ml)	3.32 ± 0.25	3.51 ± 0.22	2.51 ± 0.45	3.00 ± 0.43	3.25 ± 0.53
Yeast and mould count (cfu/ml)	10.21 ± 0.43	10.23 ± 0.34	10.65 ± 0.39	11.10 ± 0.45	11.09 ± 0.95

**Legend:** Means bearing superscript within the treatments differ significantly ( $p < 0.01$ ).

The coliform and yeast and mould count (cfu/ml) of control and treatments exhibited no significant difference among them and were within the FSSAI (2012) prescribed limits of 10 and 100 per gram (maximum) respectively.

### Sensory evaluation of yoghurt

The samples of yoghurt were subjected to sensory evaluation by a panel of six judges using the 9-point hedonic scale and scorecard adopted by Pearce and Heap (1974) and the results are presented in Table 4.

**Table 4:** Sensory properties of yoghurt samples

Parameters	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Appearance and Colour	8.42 ± 0.08	8.50 ± 0.18	8.58 ± 0.15	8.75 ± 0.17	8.83 ± 0.11
Body and Texture	8.25 ± 0.17	8.33 ± 0.17	8.50 ± 0.18	8.58 ± 0.15	8.75 ± 0.11
Flavour score	8.50 ± 0.18	8.58 ± 0.15	8.67 ± 0.11	8.75 ± 0.11	8.83 ± 0.11
Overall Acceptability	8.33 ± 0.17	8.42 ± 0.08	8.58 ± 0.15	8.67 ± 0.11	8.75 ± 0.11

The sensory scores of the control and treatments, such as appearance and colour, body and texture, flavour and overall acceptability, revealed no significant differences among them. But the sensory scores exhibited an increasing trend towards the 100 per cent substitution of cane sugar with palm sugar, indicative of the positive outcome of the experiment. Therefore, the replacement of cane sugar with palm sugar in yoghurt did not affect the acceptability based on the scores given by the sensory panel. The appearance, body and texture, flavour and overall acceptability scores exhibited in the present study are at par with the findings of Malarkannan and Geevarghese (1998) for yoghurt prepared with condensed coconut water and also in similitude with Ghadge *et al.* (2008) for apple pulp incorporated yoghurt.

### CONCLUSION

Finally, it can be concluded that the yoghurt can be prepared with palm sugar by replacing cane sugar with up to 100 per cent levels without affecting the sensory qualities. Furthermore, there was found to be no noticeable disparity in the physicochemical and microbiological analysis. The replacement of cane sugar with palm sugar in yoghurt obtained an overwhelming response from the sensory panel and hence, the palm sugar is highly preferable for the preparation of innovative yoghurt.



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# Assessment of $\lambda$ -irradiation Impact on Physiological and Sensory Characteristics of Peanut Kernels (*Arachis hypogaea* L.) as a Function of Moisture Content

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## ABSTRACT

The investigation was intended to assess the effect of  $\lambda$ -irradiation dose on physiological and sensory characteristics of peanut kernels as a function of moisture content. The peanut cultivars GG-20 and TG-37A conditioned at three initial moisture contents (6.0%, 8.5% and 11.0%), w.b. and irradiated with the different  $\lambda$ -irradiation dose (0 kGy, 2.5 kGy, 5.0 kGy, 7.5 kGy & 10.0 kGy) were stored at ambient temperature for three months. The effect of  $\lambda$ -irradiation dose on physiological parameters including germination percentage and seedling vigor (Index-I and II) and sensory qualities (colour and overall acceptability) of peanut kernels were determined. The results revealed that the  $\lambda$ -irradiation significantly ( $p \leq 0.05$ ) did adverse effect on physiological characteristics of peanut kernel. Seed viabilities in terms of germination percentage and seedling vigor of both the peanut kernels variety are negatively correlated with the  $\lambda$ -irradiation dose and the kernels' initial moisture content. At  $\lambda$ -irradiation dose 7.5 kGy, both the peanut kernels varieties lost their seed viabilities and it could not recover again during the subsequent storage period of three months. Gamma irradiation dose did not affect sensory mean score (color and overall acceptability) of peanut kernels negatively and found non-significant just after irradiation, but during storage it reported significant. Peanut kernels' initial moisture content affects sensory mean score negatively during storage.

## HIGHLIGHTS

- $\lambda$ -irradiation dose effect on physiological and sensory characteristics of peanut kernels as a function of moisture content was assessed.
- The  $\lambda$ -irradiation adversely affected physiological characteristics of the peanut kernel.
- Seed viabilities negatively correlated with the  $\lambda$ -irradiation dose and the kernels' initial moisture content.
- Gamma irradiation did not affect sensory mean score (color and overall acceptability) of peanut kernels.
- Peanut kernels' initial moisture content affects sensory mean score negatively during storage.

**Keywords:** Peanut,  $\lambda$ -irradiation, Moisture content, Physiological characteristics, Sensory quality

Groundnut or peanut, botanically known as *Arachis hypogaea* L. is one of the most important oilseed crops, globally as well as in our country which belonging to a family of Leguminosae (Hymowitz 1990). India is the second largest producer of peanut after China. The Indonesia, Vietnam Social Republic, Malaysia, Philippines and Thailand are the major export destinations of Indian peanut. Gujarat is the

leading state in production of peanut as it shares about 50% of India's total peanut production (Gojiya et al. 2020).

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Peanut (*Arachis hypogaea* L.) is one of the leading oil seed crops that produces edible oil and plant protein, as well as vitamins and minerals such as calcium, magnesium, potassium, iron and zinc. In most of the developing countries, peanut kernels are stored as dry seeds and form an enormous serve of food. However, vast quantities of seeds are lost annually as a result of microbial growth and insects attacks. The hot and humid climate of India is quite favourable for the growth of numerous insects and microorganisms that destroy stored crops and causes spoilage of food (Gowda and Ramakrishna 1997).

Gamma irradiation is a physical technique of food preservation that seems to have a potential to protect such commodity from insects infestation and microbial contamination during storage and therefore it has been proposed as a good alternative to methyl bromide and other fumigants for pest control (Loaharanu and Thomas 2001). However, development of this technique involves consideration that gamma may change the physiological and sensory properties of stored kernels. The absolute relationship of irradiation application dose and possible changes must be known in order to comprehensively assess the acceptability of irradiated peanut kernels. Considering the above facts in mind, a systematic scientific study was undertaken.

## MATERIALS AND METHODS

### Materials

Healthy and mature kernels of peanut variety GG-20 (Gujarat Groundnut-20) and TG-37A (Trombay Groundnut-37A), popular in sauratra region were procured in bulk from peanut processing industry namely Balikrut Peanut Industries, Junagadh (Gujarat). The kernels kept in Poly propylene (PP) bags for treatment and were stored at room temperature.

### Methods

#### Sample preparation

Since initial moisture content of the two varieties of peanut (GG-20 and TG-37A) kernels was high ~11% w.b., therefor be tone packaging, the peanut samples were dried at 50±2 °C to attain the safe storage

moisture level of ~6.0 % in a laboratory tray dryer (Sheppard and Rudolf 1991). The moisture content of peanuts was expressed on a wet mass basis. After determining initial moisture content, the samples were conditioned to 6.0, 8.5 and 11.0 % moisture content (w.b.) by adding predetermined amount of distilled water to the fixed quantity of kernels. The following formula was used to calculate the amount of distilled water to be added to the peanuts (Eq.-1). After addition of calculated amount of distilled water, the samples were packed in PP bags (0.05 mm thick), mixed thoroughly to ensure uniform distribution of moisture and stored in incubator at 25 °C for 24 h for moisture equilibrium (Gojiya *et al.* 2020). During the storage, the samples were stirred at every 2 h during the day time to ensure uniform moisture distribution. For each test, 10g of sample was taken out and tested for final moisture content. The moisture content of the sample was checked again using hot air oven after the equilibrium and further tests were carried out (Obi *et al.* 2014). The peanut kernels sample of ~650 g were packed in virgin poly propylene (PP) bags of 50µ thickness having size: 260 × 200 mm and sealed by hand sealing machine (Plastic Impulse Hand Sealer, model: POLY SEAL-300mm).

$$Q = \frac{Y(x-y)}{(100-x)} \quad \dots (1)$$

Where,

Q = Mass of water to be added (kg)

Y = Initial mass of sample (kg)

y = Initial moisture content of the sample (% w.b.)

x = Final (desired) moisture content of the sample (% w.b.)

#### Irradiation of peanut kernels

The healthy and mature kernels of peanut variety GG-20 (Gujarat Groundnut-20) and TG-37A (Trombay Groundnut-37A) were procured in bulk from peanut processing industry and conditioned at three initial moisture content level (6.0, 8.5 and 11.0% w.b.) as suggested by Obi *et al.* (2014) and packed in virgin Poly Propylene (PP) bags of 50µ thickness followed by sealing with hand sealing machine. These samples were irradiated at gamma radiation plant (Processing for Agro, Food and Medicine), radiation processing facility of M/s Gujarat Agro

Industries Corporation limited (GAICL), at village Bavla, Ahmedabad (a Government Enterprise) and stored at ambient temperature for three months. The samples were analyzed at every 1 month interval for quality attributes.

### Determination of physiological characteristics of peanut kernels

#### Germination percent (%)

Seed germination was determined by the wet paper method (ISTA, 1985). One hundred seeds in four replicates of 25 seeds each were placed between two layers of moistened germination paper. The germination paper was then rolled carefully avoiding any excess pressure on the seeds. These were finally wrapped in a sheet of wax paper to reduce surface evaporation and placed in a germination incubator at 25°C in an upright position in a suitable container. The seeds were evaluated after 7 days for normal seedlings, abnormal seedlings, un-germinated and dead seeds. Germination percentage was recorded on the basis of the normal seedlings only (Eq. 2).

Germination (%) =

$$\frac{\text{Number of germinated seeds} \times 100}{\text{Total number of seeds}} \quad \dots (2)$$

#### Seedling vigor

Seedling vigour is an important quality parameter which needs to be assessed to supplement germination and viability tests to gain insight into the performance of a seed lot in the field or in storage. Germinated Seedlings from each replicate were taken at random and the shoot length and root length were recorded by using a linear scale. Seedlings were dried in an oven at 80°C until constant weight and the biomass together per replicate was measured. Seedling vigor was calculated according to Abdul-Baki and Anderson (1973)

$$\text{Vigor index-I} = \text{Germination (\%)} \times \text{seedling (root + shoot) length (cm)} \quad \dots (3)$$

$$\text{Vigor index-II} = \text{Germination (\%)} \times \text{seedling (root + shoot) dry weight (g)} \quad \dots (4)$$

### Determination of sensory qualities of peanut kernels

The sensory evaluation for the organoleptic quality characteristics including colour and overall acceptability of peanut kernels was carried out according to Peryam and Pilgrim (1957) by trained panellists comprising of staff and post graduate students. Before the sensory evaluation, the panelists were subjected to a training session to familiarize them with the sensory attributes of the peanut kernel to be assessed. Each panellist was introduced to a sensory evaluation score card and briefed on the procedures of evaluation during the training. All the samples were evaluated at room temperature of about 30±1 °C under normal room light. For each test, the samples were served to the panellists in a random order. Each sample was coded with their treatment codes. The panellists were asked to give the scores for different sensory characteristics on 9-points hedonic scale.

### STATISTICAL ANALYSIS

The analysis of variance (ANOVA) of mean values generated from the analysis of each of quality attributes obtained from three replications (n=3) during the experimentation were subjected to statistical analysis using factorial completely randomized design (F-CRD) and Microsoft Excel as per the procedure suggested by Panse and Sukhatme (1985). Treatments having 0.0 kGy gamma irradiation dose (no irradiation) served as control for each variety and moisture content level.

### RESULTS AND DISCUSSION

#### Effect of Gamma Irradiation on Physiochemical Properties of Peanut Kernels

##### Germination

Germination parameter is good indicators of field performance of a seed lot. From the data it was clearly seen that, at increasing gamma irradiation dose levels and kernels' moisture content had significant effect on germination characteristics of the peanut kernels. The influence of gamma irradiation dose on germination characteristics of GG-20 and TG-37A peanut kernels at different moisture content during storage noted in Table 1. In general, as gamma irradiation dose was increased

**Table 1:** Effect of gamma irradiation on germination (%) of peanut kernels conditioned at different moisture contents during storage

Effect	GG-20				TG-37A			
	Storage period (month)				Storage period (month)			
	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
<b>Gamma radiation doses (D)</b>								
D1 (0 kGy)	85.78	75.56	71.11	71.11	87.56	75.11	71.11	72.44
D2 (2.5 kGy)	39.56	37.78	33.78	30.22	37.78	37.78	30.67	27.56
D3 (5.0 kGy)	5.67	5.33	5.67	5.33	5.33	5.78	5.78	5.78
D4 (7.5 kGy)	0	0	0	0	0	0	0	0
D5 (10 kGy)	0	0	0	0	0	0	0	0
S.Em±	0.74	0.77	0.56	0.89	0.77	0.69	0.60	0.77
CD at 5%	2.15	2.22	1.62	2.57	2.22	1.99	1.72	2.22
<b>Moisture Content (M)</b>								
M1 (6.0 % , w.b)	48.44	47.11	43.56	43.56	47.56	46.22	43.56	44.44
M1 (8.5 % , w.b)	43.11	38.22	36.00	35.11	43.56	39.11	34.67	34.22
M1 (11.0 % , w.b)	39.11	33.33	30.67	28.00	39.56	33.33	29.33	27.11
S.Em±	0.58	0.60	0.44	0.69	0.60	0.53	0.46	0.60
CD at 5%	1.66	1.72	1.26	1.99	1.72	1.54	1.33	1.72
<b>Interaction (D × M)</b>								
S.Em±	1.29	1.33	0.97	1.54	1.33	1.19	1.03	1.33
CD at 5%	3.72	3.85	2.81	4.45	3.85	3.44	2.98	3.85
C.V.%	8.54	9.73	7.65	12.50	8.84	8.70	8.32	10.92

from 0.0 to 10.0 kGy, germination percentage of the peanut kernels decreased significantly. At and above 7.5 kGy gamma irradiation dose, both the peanut kernels varieties lost their viabilities. This was true for both peanut kernels varieties and at all the three moisture contents as shown in Table 1. These results indicate that the higher doses of gamma radiation had adverse effect on seed germination. The inhibition of seed germination at higher doses of radiation may have resulted from damage to chromosomes and subsequent mitotic retardation (Al-Safadi and Simon 1996). These results are also in accordance with the findings of previous workers Bishnoi and Chandra (2014), Ripa and Audrina (1993), Agarwal and Kaul (1998) and Munishimanna *et al.* (1998), who reported that seed germination percentage decreased in different crops with increase in radiation dose. Further, in the present study, when the final germination percentage after post-irradiation storage is compared with germination percentage of those seeds tested immediately after irradiation, reduced germination percentage could

not increase again during the subsequent storage period of three months after irradiation.

### Seedling vigor

Seedling length is widely used as an index in determining the biological effects of various physical and chemical mutagens. The results of the present study showed that at increasing gamma irradiation dose and kernels moisture content levels has significant effect on seedling vigor indices of the both peanut kernels varieties (Table 2 & 3). As gamma irradiation dose was increased from 0.0 to 10.0 kGy, seedling vigor index-I and seedling vigor index-II of the both peanut variety decreased significantly. The effect of gamma irradiation dose on seedling vigor of GG-20 and TG-37A peanut kernels at different moisture content during storage presented in Table 2 and 3. Further, at and above 7.5 kGy gamma irradiation dose, both the peanut kernels varieties lost their viabilities. This was correct for both peanut kernels varieties and at all the three moisture contents. From the results it

**Table 2:** Effect of gamma irradiation on seedling vigor index-I of peanut kernels conditioned at different moisture contents during storage

Effect	GG-20				TG-37A			
	Storage period (month)				Storage period (month)			
	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
<b>Gamma radiation doses (D)</b>								
D1 (0 kGy)	696.12	581.89	546.21	547.58	560.83	450.17	451.62	448.04
D2 (2.5 kGy)	140.43	120.20	113.42	98.40	170.38	153.52	132.21	114.16
D3 (5.0 kGy)	7.86	5.74	6.81	6.74	6.72	4.63	6.04	5.26
D4 (7.5 kGy)	0	0	0	0	0	0	0	0
D5 (10 kGy)	0	0	0	0	0	0	0	0
S.Em $\pm$	4.46	5.02	6.73	7.33	3.69	3.80	5.88	6.07
CD at 5%	12.87	14.50	19.43	21.17	10.66	10.99	16.99	17.53
<b>Moisture Content (M)</b>								
M1 (6.0 %, w.b)	343.40	313.71	283.41	292.99	287.94	256.73	263.56	262.19
M1 (8.5 %, w.b)	280.96	227.30	222.68	216.60	245.81	200.39	185.56	179.66
M1 (11.0 %, w.b)	220.06	166.82	160.35	143.14	204.18	151.20	140.75	125.60
S.Em $\pm$	3.45	3.89	5.21	5.68	2.86	2.95	4.56	4.70
CD at 5%	9.97	11.23	15.05	16.40	8.26	8.51	13.16	13.58
<b>Interaction (D <math>\times</math> M)</b>								
S.Em $\pm$	7.72	8.70	11.65	12.70	6.40	6.59	10.19	10.52
CD at 5%	22.30	25.11	33.65	36.67	18.47	19.03	29.42	30.37
C.V.%	7.92	10.64	15.14	16.85	7.51	9.38	14.96	16.05

**Table 3:** Effect of gamma irradiation on Seedling vigor index-II of peanut kernels conditioned at different moisture contents during storage

Effect	GG-20				TG-37A			
	Storage period (month)				Storage period (month)			
	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
<b>Gamma radiation doses (D)</b>								
D1 (0 kGy)	19.10	16.35	15.15	14.52	17.49	14.50	14.18	14.13
D2 (2.5 kGy)	4.43	4.07	3.65	3.19	3.78	3.64	3.05	2.69
D3 (5.0 kGy)	0.30	0.29	0.29	0.28	0.27	0.28	0.29	0.29
D4 (7.5 kGy)	0	0	0	0	0	0	0	0
D5 (10 kGy)	0	0	0	0	0	0	0	0
S.Em $\pm$	0.13	0.18	0.21	0.20	0.21	0.15	0.18	0.21
CD at 5%	0.37	0.51	0.62	0.57	0.62	0.42	0.52	0.62
<b>Moisture Content (M)</b>								
M1 (6.0 %, w.b)	9.53	8.99	7.78	7.95	8.36	7.76	7.63	8.03
M1 (8.5 %, w.b)	7.83	6.55	6.38	5.67	7.17	6.00	5.54	5.16
M1 (11.0 %, w.b)	6.47	5.18	4.92	4.37	6.02	4.67	4.34	3.92
S.Em $\pm$	0.10	0.30	0.37	0.34	0.17	0.11	0.31	0.37
CD at 5%	0.29	0.88	1.07	0.99	0.48	0.33	0.91	1.07
<b>Interaction (D <math>\times</math> M)</b>								
S.Em $\pm$	0.22	0.14	0.17	0.15	0.37	0.25	0.14	0.17
CD at 5%	0.64	0.39	0.48	0.44	1.07	0.73	0.41	0.48
C.V.%	8.03	12.69	16.82	16.45	7.10	11.94	15.54	18.80

could be said that the higher doses are significantly negative effect for seedling growth (Table 2 & 3). It may be attributed to reduced mitotic activity in tissues. These results are in line with Din *et al.* (2003), Kon *et al.* (2007) and Sharma and Rana (2007). Further, in this study when the final seedling vigor after post-irradiation storage is compared with seedling vigor of those kernels tested immediately after irradiation, reduced seedling vigor could not increase again during the subsequent storage period of three months after irradiation.

**Effect of gamma irradiation on sensory quality of peanut kernels**

**Color**

The results concerning to effect of gamma irradiation dose on color mean score for both peanut varieties at different initial moisture content during complete storage period reported in Table 4. Generally, It was noticed that gamma irradiation dose (D) did not affect color mean score of peanut kernels negatively and found non-significant ( $P \geq 0.05$ ) just after irradiation, but during storage it reported

significant. It was also noticed that, as moisture content of GG-20 peanut increased from 6.0 to 11 % (w.b.), the color mean score significantly reduced in both the varieties tested. During storage gradual decrease was noticed in the color mean score of peanut kernel in control samples (non-irradiated samples) while in irradiated sample the minute change was observed. Similar results were reported by Al-Bachir (2016a and 2016b) found non-significant effect of gamma radiation treatment on flavor, color, taste and texture of pistachio nut, almond, peanut and sesame.

**Overall acceptability**

Monthly changes in overall acceptability mean score of GG-20 and TG-37A peanut kernels as influenced by different gamma irradiation dose at different initial moisture content during three month storage reported in Table 5. From the results it can be clearly seen that overall acceptability mean score of both peanut cultivar GG-20 and TG-37A was non-significantly change with respect to gamma irradiation dose during entire storage. There was of no stable pattern of change in overall acceptability

**Table 4:** Effect of gamma irradiation on color of peanut kernels conditioned at different initial moisture content during storage

Effect	GG-20				TG-37A			
	Storage period (month)				Storage period (month)			
	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
<b>Gamma radiation doses (D)</b>								
D1 (0 kGy)	7.56	6.89	6.11	5.78	7.89	7.44	6.78	6.11
D2 (2.5 kGy)	7.67	7.67	7.67	7.56	7.78	7.89	7.44	7.78
D3 (5.0 kGy)	7.22	6.89	6.89	7.22	7.33	7.00	7.11	7.33
D4 (7.5 kGy)	6.89	6.44	6.89	6.89	7.33	7.00	7.22	7.22
D5 (10 kGy)	6.89	6.44	6.56	6.67	6.89	6.44	6.89	7.22
S.Em ±	0.24	0.25	0.27	0.33	0.26	0.23	0.27	0.33
CD at 5%	NS	0.72	0.77	0.94	NS	0.67	0.77	0.96
<b>Moisture Content (M)</b>								
M1 (6.0 %, w.b)	7.87	7.53	7.53	7.67	8.13	7.80	7.87	7.93
M1 (8.5 %, w.b)	7.20	6.73	6.73	6.67	7.47	7.13	6.93	7.07
M1 (11.0 %, w.b)	6.67	6.33	6.20	6.13	6.73	6.53	6.47	6.40
S.Em ±	0.19	0.19	0.21	0.25	0.20	0.18	0.21	0.26
CD at 5%	0.54	0.56	0.60	0.73	0.58	0.52	0.61	0.75
<b>Interaction (D × M)</b>								
S.Em ±	0.42	0.43	0.46	0.56	0.45	0.40	0.47	0.58
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS
C.V.%	10.08	10.85	11.77	14.33	10.41	9.77	11.52	14.02

**Table 5:** Effect of gamma irradiation on overall acceptability of peanut kernels conditioned at different initial moisture content during storage

Effect	GG-20				TG-37A			
	Storage period (month)				Storage period (month)			
	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month	0 month (Initial value)	1 <sup>st</sup> month	2 <sup>nd</sup> month	3 <sup>rd</sup> month
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
<b>Gamma radiation doses (D)</b>								
D1 (0 kGy)	7.89	7.22	6.56	5.89	7.56	6.89	6.33	5.67
D2 (2.5 kGy)	7.56	7.22	7.22	6.78	6.78	6.89	7.00	6.22
D3 (5.0 kGy)	7.67	7.11	7.00	6.78	7.22	6.89	7.00	6.22
D4 (7.5 kGy)	7.44	6.78	6.78	6.67	7.11	6.67	7.11	6.33
D5 (10 kGy)	7.00	6.78	6.89	6.44	7.00	6.56	7.00	6.22
S.Em $\pm$	0.20	0.23	0.25	0.18	0.30	0.25	0.24	0.21
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS
<b>Moisture Content (M)</b>								
M1 (6.0 %, w.b)	8.13	7.73	7.73	7.53	7.60	7.33	7.53	6.67
M1 (8.5 %, w.b)	7.47	7.00	6.73	6.27	7.13	6.67	6.73	6.13
M1 (11.0 %, w.b)	6.93	6.33	6.20	5.73	6.67	6.33	6.40	5.60
S.Em $\pm$	0.16	0.18	0.19	0.14	0.23	0.20	0.19	0.16
CD at 5%	0.46	0.52	0.56	0.40	0.67	0.57	0.54	0.47
<b>Interaction (D <math>\times</math> M)</b>								
S.Em $\pm$	0.35	0.40	0.43	0.31	0.52	0.44	0.42	0.37
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS
C.V.%	8.18	9.96	10.82	8.25	12.54	11.21	10.60	10.31

mean value was noticed in both peanut varieties during storage. Similar results were reported by Al-Bachir (2015a, 2015b, 2016a and 2016b) found non-significant effect of gamma radiation treatment on flavor, color, taste and texture of pistachio nut, almond, peanut and sesame. During three month of storage, overall acceptability mean value reduced regularly in control samples (non-irradiated samples) while in irradiated sample the negligible change was reported in peanut kernels. These results are in agreement with previous findings of Rao and Vakil (1985) in legumes (green gram, lentil, horsebean and Bengal gram), Ocloo *et al.* (2011) for cowpea seed, Al-Bachir (2016b) for peanut seeds, Hyun *et al.* (2004) for kimchi.

## CONCLUSION

The present investigation inferred that gamma irradiation significantly ( $p \leq 0.05$ ) affect physiological characteristic of peanut kernels. As gamma irradiation dose was increased from 0.0 to 10.0 kGy, seed viabilities in terms of germination percentage and seedling vigour of both the peanut

kernels variety decreased significantly. At gamma irradiation dose at and above 7.5 kGy, peanut kernels of both the varieties lost their seed viabilities. This was true for both the varieties and at all the three moisture contents. Moreover, reduced seed viabilities of kernels of both the peanut varieties with different initial moisture content could not increase again during the subsequent storage period of three months after irradiation. In addition to that gamma irradiation dose did not negatively affect sensory mean score of peanut kernels and found non-significant just after irradiation. But during storage it was observed significant. As moisture content increased, the sensory mean score of kernels of both the peanut varieties significantly decreases in both the varieties.

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# Efficiency and Effectiveness of Mutagenic agents (Gamma rays and Ethyl Methane Sulphonate) on *Bougainvillea* spp.

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## ABSTRACT

Mutation breeding is a well-known crop improvement technique that has resulted in the development of many new decorative flower shape and color mutant types. In order to develop originality between 2020 and 2021, researchers at the College of Horticulture in Bengaluru, Karnataka, India, employed a physical mutagen like gamma rays and a chemical mutagen, Ethyl Methane Sulphonate to induce mutations in *Bougainvillea* spp. Hardwood cuttings were treated to three different concentrations of Ethyl Methane Sulphonate as well as four different gamma-ray dosages (5, 10, 15, and 20 Gy) (0.6, 0.8 and 1.0 percent). Several morphological features were assessed in the first mutant-vegetative (M1V1) generation. As the mutagen dose concentration increased, so did the percentage of sprouting seedlings and the survival rate. All morphological attributes have been recorded a declining trend with increasing mutagenic treatments. Albina, albina green, xantha, chlorina, viridis, yellow viridis, striata, maculata, and variegated chlorophyll mutations were discovered. Chlorina was the most common chlorophyll mutant identified, followed by maculata and viridis. When it came to mutagenic treatments, EMS 1.0 percent had a higher number of morphological mutants (3) as well as chlorophyll mutants (6 Nos.). Gamma-radiation at 5.0 Gy had a higher mutagenic effectiveness (212.43) and efficiency (112.69) than the other treatments. In terms of efficacy (112.23) and efficiency, EMS produced the highest mutation rates in *Bougainvillea* (87.65).

## HIGHLIGHTS

- Mutation breeding.
- Mutagen like gamma rays and a chemical mutagen, Ethyl Methane Sulphonate.
- Different gamma-ray dosages (5, 10, 15, and 20 Gy) EMS concentrations (0.6, 0.8 and 1.0 percent).
- Variegated chlorophyll mutants.
- Mutagenic effectiveness and efficiency.

**Keywords:** Mutagenicity, mutation, mutant-vegetative generation, *Bougainvillea*, mutagens

*Bougainvillea* is a well-known ornamental plant that can be found in tropical and subtropical gardens around the world. *Bougainvillea*'s popularity originates from its bright bracts and ability to adapt to a wide range of agro-climatic conditions. Because it is a popular plant in the horticultural nursery trade and is grown in a variety of gardens, it would benefit from more colour, size, and shape variation. For many years, mutagenic treatments have been used in plants to promote desirable

phenotypic variation. Plant spontaneous and induced mutations aid in genetic dissection of the function of wild type genes. Any crop's genetic variability must be evaluated in order to establish

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a successful breeding programme. As a result, the genetic variety produced by induced mutations can undeniably aid in the recovery of alleles for increased productivity and improved plant type. Induced mutations have been used to improve crop plants, resulting in a number of mutants that have been adopted as new cultivars (Gottschalk and Wolf 1983). In India, modifying the plant type produced 18% of the varieties formed through mutation breeding (Jain, 2010).

## MATERIALS AND METHODS

The experiment was conducted at the Department of Floriculture and Landscape Architecture, College of Horticulture, University of Horticultural Sciences Campus, GKVK, Bengaluru, during 2020-21, to see how different doses of gamma-rays and Ethyl Methane Sulphonate (EMS) affected Bougainvillea sprouting, survival, and growth related traits. Based on the fatal dose of cultivars, three treatments with each mutagen were employed for the population of M1 V1 generation. As biological material, each treatment used around 300 hardwood cuttings. The hardwood cuttings were subjected to three different doses of gamma-irradiation: 5, 10, 15, and 10 Gy, with untreated cuttings serving as a control. Cobalt-60 was used as the radiation source for the gamma-irradiation (BRAC, Trombay, Mumbai).

**EMS therapy:** Three different concentrations were used for EMS treatment, namely 0.6, 0.8, and 1.0 percent for 4 hours in each concentration, with water soaked (2 hrs) cuttings serving as a control. The hardwood cuttings were pre-soaked in water for 1 hour to increase the tissues' ability to absorb the EMS. After drying completed in the shade, the water-soaked cuttings were immersed in freshly prepared EMS solutions for 4 hours after that thoroughly washed with water for one hour to eradicate chemical residues.

To classify chlorophyll mutations, the Gustafson (1940) and Blixt (1940) systems were utilised (1972). In the M1 V1 population, a wide range of morphological and chlorophyll mutations were studied. The formulae developed by Konzak *et al.* (1965) were used to calculate mutagenic efficacy and efficiency by summing the mutation frequency data recorded for each mutagenic treatment.

Mutagenic effectiveness =

$$\frac{\text{Mutagenic Frequency}}{\text{Dose or (Concentration} \times \text{time)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutagenic Frequency}}{\text{Biological damage}}$$

Mutation rate =

$$\frac{\text{Sum of values of efficiency or effectiveness of particular mutagen}}{\text{No. of treatments of a particular mutagen}}$$

Mutation rate (MR) provides the knowledge of mutations induced by a particular mutagen irrespective of dose or concentration.

## RESULTS AND DISCUSSION

According to Gaul, physical and chemical mutagens induce physiological harm (injury), gene mutations (point mutation), and chromosomal aberrations (chromosomal aberration) in the M1 generation (1970). The quantitative determination in M1 could help forecast mutagen efficacy and identify desired mutants. Because seedling height and survival have been linked to M1 mutation frequency, evaluating M1 injury using these measures is a conventional strategy in mutant breeding (Etsuo Amano, 2004). The effect of mutagenic treatments on M1 production was assessed as a percentage reduction in sprouting relative to the control. In the current study, the reduction in sprouting percentage and survival percentage (60 DAP & 120 DAP) of treated cuttings was concentration dosage dependent and linear (Table 1). Control sprouting rates of 93.00 percent and 94.90 percent were observed for gamma-rays and EMS, respectively. Among the treatments, 5.0 Gy of gamma-irradiation and 0.8 percent EMS resulted in higher sprouting rates of 85.70% and 88.49%, respectively. In terms of survival percentages, the gamma-ray treatment achieved a maximum of 89.76 percent (control) and 62.50 (5 Gy), while the EMS treatment achieved 91.56 percent (control) and 73.34 (0.6 percent) during 60 DAP and 120 DAP, respectively. Following gamma-ray exposure, there was a significant reduction in sprouting and survival percentages.

**Table 1:** Effect of mutagens on sprouting and survival percentage of *Bougainvillea* spp.

Treatments	S %	ROC	(60DAP) Survival %	ROC	(120DAP) Survival %	ROC
<b>Physical mutagen (gamma rays)</b>						
Control	93.00	—	89.76	—	84.50	—
5 Gy	85.70	8.66	62.50	32.43	58.45	40.50
10 Gy	72.40	21.50	57.45	37.50	52.12	44.20
15 Gy	65.30	31.50	50.56	34.67	43.67	51.26
20 Gy	55.40	39.40	42.78	48.98	41.35	11.89
<b>Chemical mutagen (EMS) with 4hr treatment</b>						
Control	94.90	—	91.56	—	89.56	—
0.6%	87.20	5.34	73.34	22.34	73.23	27.45
0.8%	88.49	12.67	65.34	30.76	62.45	33.30
1.0%	76.65	18.65	58.46	35.23	52.45	41.45

S: Sprouting (%); ROC: % reduction over control.

**Table 2:** Effect of mutagens on growth related traits in M1V1 generation of *Bougainvillea* spp.

Treatments	SL		IL		NSC		NL		AL		LCC	
	Mean ± SE	CV. (%)	Mean ± SE	CV. (%)	Mean ± SE	CV. (%)	Mean ± SE	CV. (%)	Mean ± SE	CV. (%)	Mean ± SE	CV. (%)
<b>(gamma rays) (Gy)</b>												
Control	20.45 ± 0.63	8.16	1.23 ± 0.34	24.12	2.9 ± 0.42	15.09	22.41 ± 0.34	11.34	—	—	32.09 ± 0.23	14.34
5 Gy	18.23 ± 0.45	102.32	1.45 ± 0.32	41.23	1.42 ± 0.23	43.23	8.84 ± 0.23	43.56	23.45 ± 0.34	82.34	28.34 ± 0.78	23.67
10 Gy	16.21 ± 0.21	123.56	2.34 ± 0.12	32.12	1.51 ± 0.63	32.43	5.67 ± 0.33	22.54	36.78 ± 0.25	76.55	37.55 ± 0.78	33.21
15 Gy	18.23 ± 0.15	98.23	1.34 ± 0.32	36.78	1.52 ± 0.95	30.56	11.34 ± 0.12	34.67	38.56 ± 0.78	48.76	35.53 ± 0.34	16.56
20 Gy	4.34 ± 0.23	87.53	1.45 ± 0.21	44.56	1.24 ± 0.35	28.56	8.23 ± 0.14	45.76	39.56 ± 0.55	32.55	38.67 ± 0.22	21.59
<b>Chemical mutagen (EMS) with 4hr treatment</b>												
Control	27.34 ± 0.56	10.04	1.32 ± 0.21	16.45	3.11 ± 0.22	22.34	34.8 ± 2.32	19	—	—	28.65 ± 0.87	8.77
0.6%	11.34 ± 0.32	62.16	1.08 ± 0.37	34.56	2.35 ± 0.45	42.58	23.5 ± 0.65	43.34	34.56 ± 2.89	52.76	28.54 ± 0.34	12.78
0.8%	8.89 ± 0.21	68.97	1.00 ± 0.01	34.89	2.56 ± 0.34	42.67	24.76 ± 1.13	42.43	27.56 ± 1.65	32.54	31.59 ± 0.33	15.88
1.0%	6.78 ± 0.21	90.45	0.98 ± 0.8	29.76	2.5 ± 0.01	35.08	16.76 ± 0.56	46.67	31.78 ± 1.78	28.45	35.35 ± 0.45	18.55

SL: Shoot length (cm); IL: Internodal length (cm); NSC: Number of sprouts cutting-1; NL: Number of leaves; AL: Abnormal leaves (%); LCC: Leaf Chlorophyll content (mg g<sup>-1</sup>); CV: Coefficient of Variation; SE: Standard Err.

Datta and Banerji (1997) found similar results in four double bracted *Bougainvillea* cultivars, as did Gupta and Shukla (1974) in nine *Bougainvillea* cultivars. Survival was reduced following gamma-ray exposure due to inactivation and/or decrease in auxin content, which impacts cell division and ultimately results in poor establishment and survival (Gordon 1957; Mahure *et al.* 2010) or deadly effect of gamma-rays produced by chromosomal abnormality (Datta and Banerji 1993).

According to Diltal *et al.* (2003), higher doses of EMS lowered plant survival in chrysanthemum. Misra and Bajpai (1983a) found that different chemical mutagens, such as EMS, DES, and MNH, reduced the survival rate of gladiolus plants by up to 50%. The severe drop in plant survival may be attributed to the creation of some toxic compounds by specific biochemical processes, which cause cell death, resulting in plant mortality (Sax 1955; D'Amato and Ostenhof 1956; Gordon 1956). Mean growth-related trait performance Table 2 shows data on the reaction

**Table 3:** Mutagenic effectiveness and efficiency based on chlorophyll mutations in the M1V1 generation of *Bougainvillea* spp.

Treatments	SVR	MF	E	E <sup>+</sup>	MRTE	MRTE
<b>Physical mutagen (gamma rays)</b>			<b>M</b>	<b>M<sup>*</sup></b>		
Control	—	—	—	—	134.67	63.34
5 Gy	9.78	10.34	212.43	122.56		
10 Gy	20.78	9.23	123.67	56.78		
15 Gy	28.76	8.96	96.43	43.23		
20 Gy	30.45	8.88	90.23	37.54		
<b>Chemical mutagen (EMS) with 4hr treatment</b>						
Control	—	—	—	—	156.67	67.54
0.6%	5.45	4.56	112.23	67.65		
0.8%	12.43	9.87	156.78	74.23		
1.0%	19.54	11.56	201.34	56.56		

SVR: % survival reduction (L); MF: Mutation frequency (M); E: Effectiveness; E<sup>+</sup>: Efficiency; M: (M×100) Gy<sup>-1</sup> or (C×t); M<sup>\*</sup> (M×100) L<sup>-1</sup>; MRTE: Mutation rate in terms of Effectiveness; MRTE\* Mutation rate in terms of Efficiency.

of several agronomical features to applied doses of the mutagen, both physical and chemical. The statistics show that there are variances in character values that vary from treatment to treatment. It was discovered that under all treatments, the values for the majority of the characteristics decreased with increasing concentration. The length of the shoot ranged from 1.42 cm (5.0 Gy) to 4.51 cm (15.0 Gy). Shoot length rose marginally more at higher doses (10.0 Gy) than at lower doses of mutagenic therapy. Kainthura and Srivastava (2015) in tuberose and Ramesh *et al.* (2012) in mulberry both reported an increase in plant height with increasing mutagen dose to an optimum level of mutagenic dose. According to Fowler and Mac Queen (1972), the majority of the observed stimulatory effects of modest doses of radiation were caused by early changes in axillary bud development and variations in the initial pace of floral differentiation.

There was a progressive and significant decrease in the values with increasing concentration in EMS treatments, indicating a negative shift. Gamma-rays had a smaller shoot length than the EMS treatment of the two mutagens. Internodal length increased proportionally with increasing dose of gamma-rays, ranging from 1.22 cm (5.0 Gy) to 1.34 cm (10.0 Gy) in gamma-ray treatments, whereas it decreased proportionally with increasing concentration, ranging from 0.93 cm (1.0 percent) to 1.05 cm (0.8 percent) in EMS treated cuttings. Among all treatments, EMS had the shortest internodal length (1.0 percent). There was little change in the quantity

of sprouts between the gamma-ray treatments. The similar thing was observed in EMS treatments.

Treatments with both physical and chemical mutagens resulted in fewer leaves with increasing mutagen dose, which is consistent with the findings of Roychowdhury and Tah (2011) in carnation. Vegetative anomalies such as diversity in leaf shape, size, margin, and apex fusion increased dramatically in *Bougainvillea* as a result of the influence of several mutagenesis treatments. The frequency of anomalies increased as mutagen doses rose. Among all treatments, 10.0 Gy of gamma-radiation produced the most aberrant leaves (45.05%) and 1.0 percent EMS (35.49%) when compared to other EMS doses.

It could be attributed to inactivation or disruptions in auxin synthesis (Gordon, 1957), as well as the amount of chromosomal abnormalities (Sparrow and Evan, 1961). Similar evidence has been reported in *Bougainvillea* by Datta and Banerji (1997), Gupta and Shukla (1974), and chrysanthemum by Misra *et al.* (2009) and Kapadiya *et al.* (2009). (2014). In terms of leaf length, both -ray treatments and EMS treatments reported lower values as the mutagen dosage increased.

In gamma-ray treatments, 5.0 Gy produced the greatest mean value of leaf length (3.31 cm), whereas 0.8 percent produced a greater value of 3.58 cm. Except for 1.0 percent EMS, which recorded 3.20 cm due to the mutagenic effect, other mutagenic treatments showed a steady decrease in leaf width values. Leaf thickness increased with increasing



dosage in both mutagens in all of the mutagen-treated populations. Kumari *et al.* (2013) found that greater doses of gamma-rays reduced leaf size in terms of length and width in the chrysanthemum cultivar 'Otome Pink'. Variability in morphological characteristics The co-efficient of variation, which measures induced variability, revealed highly significant variations between treatments (Table 2).

In terms of shoot length (123.56 percent), leaf length, and leaf thickness, the coefficient of variation was highest at 10 Gy gamma-rays compared to other treatments. 5.0 Gy had the most variability for the attributes number of sprouts (42.23 percent) and aberrant leaves percentage (82.34 percent) among all gamma-treatments. When compared to the other two treatments, 10.0 Gy of gamma-radiation produced the largest co-efficient of variation in internodal length and leaf number, with 41.23 percent and 43.56 percent, respectively. In the case of EMS-treated cuttings, the 0.8 percent concentration resulted in larger changes for many of the morphological features, including internodal length (34.89 percent), number of sprouts (42.67 percent), and aberrant leaf percentage (52.76 percent), than the other concentrations.

In terms of shoot length variability, EMS exhibited a higher variability of 90.45 percent than previous EMS treatments. When all treatments of the two mutagens were considered, gamma-rays displayed the most diversity for the phenotypes identified.

This was supported by the cv. % recorded for all morphological features, which were much greater than the control. Padmadevi (2009) observed similar results in chrysanthemum and Mekala (2009) in jasmine for different growth related features. Gregory (1966) proposed that the shift in mean in many of the treated populations was due to differences in the degree of induced individual changes in M1 V1 production with both physical and chemical mutagenic treatments. Among the treatments, 1.0 percent EMS produced the most (10) morphological mutants, including seven dwarf mutants and five thornless mutants. Each of the 5 Gy and 10.0 Gy gamma-rays detected one early flowering mutation.

Padmadevi (2009) discovered miniature and early flowering mutants in chrysanthemum using several mutagenesis techniques. Chlorophyll mutants In the

current study, nine different types of chlorophyll mutants were documented in M1 V1 generation for varied gamma-ray and EMS dose concentrations. Chlorophyll mutation spectrum in the M1 V1 generation Albina, albina green, xantha, chlorina, viridis, yellow viridis, striata, maculata, and variegated types are among them. Albina mutants lacked chlorophyll and could only survive for a few days. The leaves of Albina-green and Yellow viridis were green white and yellow green, respectively.

Xantha possessed pale yellow leaves, and these mutants did not live long due to chlorophyll disruption. The 10 Gy gamma-rays treatment produced the most types of mutants (6), followed by the 5.0 Gy and 1.0 percent EMS treatments, both of which produced five types of chlorophyll mutants. Among the mutagens, EMS produced more chlorophyll mutants than gamma-ray treatments.

Chlorina was the most common mutant found, followed by maculata and viridis. Chlorina was present in all mutagen doses and was produced at the highest frequency in all mutagenic treatments, whereas albina green, yellow viridis, and variegated type mutants were infrequently induced and expressed at a lower frequency. Albina was discovered in gamma-rays with a low frequency.

Kolar *et al.* (2011) discovered 11 different forms of chlorophyll mutants in *Delphinium malabaricum* (Huth) Munz. using diverse mutagenesis procedures. Bhattacharya (2003) found a higher frequency and a broader spectrum of chlorophyll mutants in chemical mutagen EMS in carnation. The mutagenic frequency and dose levels were used to determine mutagenic effectiveness (Table 3). Mutagenic effectiveness was shown to be higher (215.2) in gamma-irradiation at 5.0 Gy, whereas it was higher (205.83) in EMS treatment at 1.0 percent concentration.

The findings of Roychowdhury and Tah (2011) on the mutagenic effectiveness of EMS in carnation are consistent with the findings of this investigation. Mutagenic efficiency was shown to be inversely related to mutagen dose. The highest was 212.43 in the 5.0 Gy gamma-ray treatment and 74.23 in the 0.8 percent EMS. Padmadevi (2009) found similar results of mutation efficiency with lower doses of gamma-ray in chrysanthemum.



Since the mutagens were found to be both effective and efficient in this investigation. Mutation rates were also computed. Efficient mutagenesis is the generation of desirable alterations with the least amount of unwanted side effects. A high mutation rate paired by minimum negative effects is desirable in a mutation breeding programmer. However, in general, the mutagen that causes a higher mutation rate also causes a high level of mortality, sterility, and other negative outcomes (Blixt *et al.* 1964).

## CONCLUSION

Inducing dwarfness and thornlessness with 1.0 percent EMS is more effective than other mutagenic treatments, although earliness in blooming was achieved with 5 Gy and 10.0 Gy gamma-rays. Among the gamma-ray and EMS treatments, the higher dose of EMS (1.0%) was more successful because it generated a greater number of chlorophyll mutations, followed by 0.8 percent EMS and 5.0 Gy gamma-rays. Chlorina mutants emerged more frequently in all treatments than other forms of chlorophyll mutants.

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# Influence of Ozone Treatment on Carbohydrate Content of Wheat (*Triticum aestivum*) during Bulk Storage

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## ABSTRACT

The present study aimed to understand gaseous ozone exposure time and frequency of ozone cycle effect on carbohydrate content of wheat variety- GW 496 during storage, this experiment was conducted. The bulk storage of wheat grain in metal silo have major issue of insects and pests which is controlled by gaseous ozone treatment. Ozone gas is the acceptable and economically viable technique for treating grains during storage for its residue-free and environment-friendly nature. In this research article, the ozone gas treatment given to the wheat grain during bulk storage and evaluated its influence on wheat carbohydrate content. A pilot-scale ozone disinfestation system for wheat grains was developed. The two-factorial experimental design on the influence of the parameters of the technological process of ozone treatment on the physicochemical qualities of wheat seeds was carried out. Wheat grains were treated by gaseous ozone with various time durations (0 min, 30 min, 60 min, 90 min and 120 min) and at various frequency cycles (7 days, 14 days, 21 days). Based on the experimental data, the effect of ozone treatment time and the ozone frequency cycle on the carbohydrate percentage of wheat grain was observed. There is negligible effect of normal dose of ozone on carbohydrate content of wheat grain during storage. On the other hand, excess ozone can also cause some negative effects on carbohydrate. This study provided new insights into how stored wheat grain responds to ozone treatment and highlighted the role of treatment time durations and frequency of cycle for wheat physicochemical property.

## HIGHLIGHTS

- **Ozone:** An environment friendly alternative of all chemicals used in bulk storage of Grains. Successful pest and insect control during storage. Scientists, grain processors, farmers, and seed producers are currently enamoured with ozone gas as a non-chemical alternative for protecting stored grains.

**Keywords:** Ozone, wheat, carbohydrate, bulk storage, ozone exposure time, ozone cycle

The storage of grains plays an important role in country's economy. The storage of grains is practiced from the age of the start of civilization. Wheat is the second most staple food crop of India after paddy. Wheat contributes nearly one-third of the total food grain production. India holds second rank among the wheat growing countries of the world. As per

GAIN Report USDA, India is heading for third consecutive record wheat harvest with marketing

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year (MY) 2019/20 (April/March) production forecast at 100 million metric tons (MMT). Largest wheat growing states in India are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar and Gujarat. Maintaining threshold temperature, proper humidity within the storage environment are the important problems faced in bulk storage structure. Main objective of bulk storage is to prevent stored grain from deterioration of quality. Insects consume large amounts of stored grain and cause other deteriorating factors that will affect the economic value of the stored grain. Due to the infestation of insects and pests, the reduction in carbohydrate content of wheat grain occurs. Global population growth inevitably results in increase in food needs. At the identical time, agricultural land has been decreased drastically because of the rapid urbanization and industrialization which severely affects the land availability for the growers. The traditionally followed chemical methods for insecticide during storage have major limitations including being environmentally unhealthy, time-consuming, and are labor-intensive.

Ozone is a strong oxidant that has been widely utilized in gas form and mixed with water as a decontaminant in the food and beverages, medical and water supply industry. Ozone is used as an alternative for pest control and reduction of microorganisms including mold and mycotoxin in stored grain (Mendez *et al.* 2003). Artificially produced ozone can decompose rapidly therefore it cannot be accumulated or transported. Thus, it should be continuously generated (Miller *et al.* 1978). Ozone may easily be generated onsite where it's required, by either corona discharge (CD), ultraviolet (UV) or electrolysis of water (Kim *et al.* 1999). Chemical insecticides give more production compared to control treatment (Kumar *et al.* 2021) but have many side effects after consuming. Ozone offers several safety advantages over chemical pesticides. There are no repositories of toxic chemicals, nor risks of residual pesticides from chemical mixing or disposal, additionally no packaging waste is produced. Ozone has a short half-life so that it reverts to naturally occurring oxygen and does not leave any residue on the product. The main objective of this study was to determine the effect of ozone on the total carbohydrate content of ozone-treated bulk stored wheat. But the chemical

insecticide has its own disadvantages on human body. So ozone is best preferred to prevent losses during storage of wheat grains.

Wheat's economic importance and contribution to human diets cannot be denied. Wheat is often viewed primarily in terms of energy as a carbohydrate and certainly plays a vital role in that respect. The grain is also rich in protein and fiber as well as lipids, vitamins, minerals and phytochemicals which may promote a healthy diet. Cereals and breads are the main source of energy for all age groups. Including sugar, starch, and fiber in the total carbohydrate content is subject to the labeling of carbohydrates as a whole. The body prefers carbohydrates and starches for energy due to the ease with which they can be metabolized into glucose, or blood sugar, in the body. The wheat grain has 85 percent (w/w) carbohydrate at maturity, with 80 percent of that being starch (endosperm) and approximately 12% cell wall polysaccharides. (Stone and Morell, 2009). Nandini and Salimath (2001) found that wheat flour contained 84.5% carbohydrate and 10% protein and 58% starch. Fraser *et al.* (1956) determined total carbohydrate content 71.6%. Kumar *et al.* (2011) studied on wheat contents and suggested that wheat contains carbohydrate 78.10%, protein 14.70%, fat 2.10%, minerals 2.10% and considerable proportions of vitamins (thiamine and vitamin-B) and minerals (zinc, iron). Wheat is also a good source of trace minerals like selenium and magnesium, nutrients essential to good health. Imran *et al.* (2013) worked on proximate analysis of indigenous wheat varieties (g/100g). They noticed approximately 73% carbohydrate content.

Population studies have shown that eating whole grains reduces the risk of serious, diet-related diseases. The coronary heart disease, cancers, inflammatory bowel disease and disordered laxation fall under these categories. Whole grains provide valuable health benefits through their carbohydrate content. Soluble NSP (non-starch polysaccharides) lower plasma cholesterol and so can reduce heart disease risk but the effect is inconsistent. There is considerable potential for cereal grains to promote large bowel health through their complex carbohydrates (Topping 2007). Wheat is also a good source of trace minerals like selenium and magnesium, nutrients essential to good health (Kumar *et al.* 2011).



Clegg (1956) worked on estimation of carbohydrate. A colorimetric method used for estimation of carbohydrate by anthrone reagent for soluble sugars in cereals, cereal by-products. Fraser *et al.* (1956) determined carbohydrate by two different methods like 'by difference' and 'by parts'. They compared the result of both method. The result of 'By difference' method reported that moisture content 14%, ash content 0.9%, Protein 11.6% and Fat 1.9%. The total of these content was 28.4% and the remaining content was carbohydrate which was 71.6%. The 'by parts' method given starch 67.4%, sugar 1.3%, pentosans 2.4%, cellulose 0.6%. The total of these all contained 71.7% total carbohydrate content. Sandhu (2012) estimated total carbohydrate content which determined by phenol/sulfuric acid assay. In the study carried out by Masuko *et al.* (2005), the carbohydrate content of grains was quantitatively estimated by phenol sulphuric acid. Phenol sulphuric acid method is the most reliable and easiest method among the quantitative assays for carbohydrate estimation. This method is widely used to determine the total concentration of carbohydrate present in foods (Roberts and Elias 2011).

Oessoe *et al.* (2014) worked on long-term rice storage and reported changes in the physical, chemical and functional quality of rice. They noted that changes in carbohydrates lead to damage. Degradation of carbohydrates into CO<sub>2</sub> during the storage process usually happens very slowly, except when the humidity reaches 14%. Decreased levels of carbohydrates occurred on red rice (52.23%) during six-month storage. Rehman and Shah (1999) reported that total soluble sugars increased by 12% after six months of storage.

Sandhu *et al.* (2012) reported that ozone treatment elevates levels of carboxylic groups and decrease total carbohydrate content in the amylopectin fraction. As a result of ozone treatment of flour and isolated starch, there was partial depolymerization of high molecular weight amylopectins, which caused the production of low molecular weight starch polymers and amylose. Visual analysis via scanning electron microscopy indicated no effects from ozone treatment. Similarly, X-ray diffraction and change in gelatinization from DSC both indicate that starch granule crystallinity was not affected by ozone treatment.

The majority of research has focused on oxidation of starch from corn, tapioca, rice, sago and waxy starch using ozone (An & King, 2009; Chan *et al.* 2009). There have been few studies covering the use of ozone as an oxidant for wheat starch. Therefore, the purpose of the study was to determine how ozone treatment affects the total carbohydrate content of wheat.

## MATERIALS AND METHODS

The freshly harvested cleaned wheat grains (GW 496) were collected from Junagadh, Gujarat, India. The unwanted materials present in wheat grains, if any, were removed. The samples having an initial moisture content of 10.40% (wb). The moisture content was determined using the AOAC-935.29 method.

In the bulk storage, the wheat grain samples (20 kg) were filled in bins and subjected to different ozone exposure time (30, 60, 90, 120 min) and ozone frequency cycle (7, 14, 21 days). The conditions, at which maximum mortality was achieved, were considered for the final treatment.

The wheat seed carbohydrate was measured using phenol sulfuric acid method. The phenol sulfuric acid method is the colorimetric method and is widely used to determine the total concentration of carbohydrates present in foods. In a test tube, phenol and sulfuric acid are added along with a clear aqueous solution of carbohydrates to be analyzed. In a test tube, phenol and sulfuric acid are added along with a clear aqueous solution of carbohydrates to be analyzed. After interacting with the carbohydrates and the phenol, the solution turns yellow-orange. It is important to note that the absorbance at 420 nm is proportional to the initial concentration of carbohydrate in the sample. Sugars that are not reducing are converted to reducing sugars by sulfuric acid, which is why this method is used to determine the total sugar content of the solution. Due to the non-stoichiometric nature of this method, a calibration curve must be prepared with standards of a known carbohydrate concentration.

## Statistical analysis

For determination of accurate effect of ozone on seeds, two factorial experiment was established. All observation of total carbohydrate percentage were analyzed using analysis of variance (ANOVA).

The main results were expressed in terms of mean standard deviation and  $p < 0.05$  were considered statistically significant.

## RESULTS AND DISCUSSION

In laboratory of CAET, Junagadh Agricultural University, Junagadh, an experiment was set to identify the effect of various time of exposures of ozone and frequency cycle of ozone on bulk storage of wheat. Monthly 26 wheat samples were collected and analysed for carbohydrate content of wheat during storage.

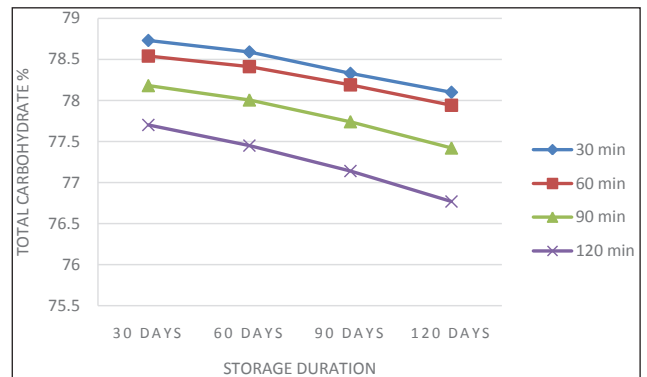
**Table 1:** Influence of ozone on total carbohydrate content of stored wheat (%)

	Storage period (Total carbohydrate %)			
	30 days	60 days	90 days	120 days
Control	78.94	78.88	78.76	78.75
<b>Exposure time(T)</b>				
30 min	78.73	78.59	78.33	78.10
60 min	78.54	78.41	78.19	77.94
90 min	78.18	78.005	77.74	77.42
120 min	77.70	77.45	77.14	76.77
CD	0.401	0.448	0.382	0.317
SEM	0.129	0.144	0.123	0.102
<b>Ozone Cycle (C)</b>				
7 days	77.95	77.68	77.30	76.89
14 days	78.34	78.18	77.93	77.61
21 days	78.57	78.47	78.39	78.18
CD	0.347	0.388	0.331	0.274
SEM	0.111	0.176	0.106	0.088
<b>T*C</b>				
CD	N/A	N/A	N/A	N/A
SEM	0.223	0.249	0.212	0.176
C.V.%	0.342	0.380	0.352	0.310

The result of the experiment on the effect of ozone gas on the total carbohydrate percentage of wheat seeds of GW 496 is presented in Table 1. It can be seen from Table 1, there was a significant difference ( $p < 0.05$ ) in the total carbohydrate percentage of untreated wheat grains and ozone-treated samples. A decrease from (78.94% to 78.75%) in total carbohydrate percentage was observed for the control sample for the storage period of 30 days to 120 days whereas a decrease from (78.73 to 76.77 %) in total carbohydrate percentage was observed in ozone-treated sample for the storage period of 30 days to 120 days. Sandhu *et al.* (2012)

found a similar trend in the storage of wheat and they recommended ozone treatment. The loss in total carbohydrate content of sample may be attributed to the hidden infestation in addition to grain's own metabolic activity. There might be some eggs, which hatched over the period of storage and damaged grain over the period of storage and at room temperature.

From the result, ozone exposure time significantly affects the total carbohydrate percentage of wheat grains. From 30 min to 60 min of exposure of ozone to wheat grains in a bin negligibly affects its total carbohydrate percentage. If ozone exposure time increase after 60 min then total carbohydrate percentage starts to decrease and 90 min and 120 min of exposure time of ozone led to decrease the total carbohydrate percentage. The reduction rate of total carbohydrate after 60 min is more than control because of lethal effect on insect and infestation is under control. The lower carbohydrate content is attributed to oxidation of sugars and/or to partial depolymerization of amylopectin chains (Kesselmans *et al.* 2004; Murphy 2000). So, exposure time has significant effect on total carbohydrate. The similar result was reported by Sandhu *et al.* (2012) that decrease in total carbohydrate content in amylopectin fractions with ozone treatment.



**Fig. 1:** Effect of ozone exposure time (min) on total carbohydrate percentage of wheat during 4 month of storage

As shown in Fig. 1, ozone exposure time at 30 min have higher total carbohydrate percentage all over the storage period of 120 days compare to other treatments. Ozone exposure duration is the main factor in oxidation of starch inside grain. The total carbohydrate percentage in starting of storage negligibly reduced with ozone application for up to 60 days and then total carbohydrate percentage decreased with the increase of storage duration.



Thus, 30 min exposure time gives the best result of total carbohydrate content during storage of 120 days. Here from Fig. 1, 120 min ozone exposure duration has negatively affect on the carbohydrate percentage of wheat grain. High ozone exposure doses deteriorate the physicochemical properties of wheat grain.

The effect of ozone cycle at the frequent time on total carbohydrate percentage is significant. From 7 days frequent cycle to 14 days frequent cycle of ozone, total carbohydrate percentage increase and at 21 days cycle of ozone it gives highest total carbohydrate percentage then above both cycles. So, 21 days cycle gives best result as compared to 7 and 14 days cycles. The reason behind this is frequently high exposure to ozone decrease the total carbohydrate percentage due to oxidation.

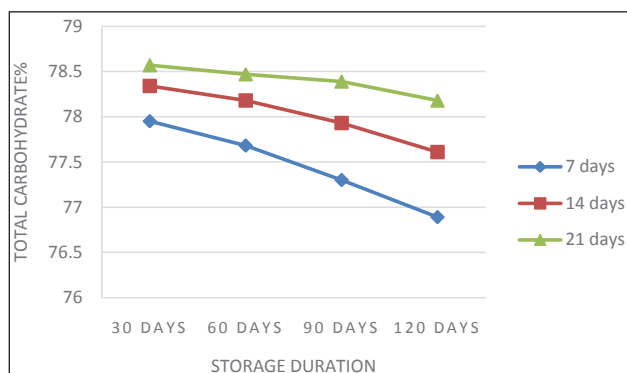


Fig. 2: Effect of ozone cycle (days) on total carbohydrate percentage of wheat during 4 month of storage

As shown in Fig. 2, ozone cycle of 21 days provides higher total carbohydrate percentage compared to 7 days and 14 days of ozone cycle treatment over the storage period of 120 days. Total carbohydrate percentage is fallen down cautiously with storage period up to 60 days storage period at regular ozone cycle. After 60 days of storage, total carbohydrate percentage is fallen down rapidly with continuous ozone applied cycle. The reason behind reduction in carbohydrate is that the higher ozone frequency adversely affects the total carbohydrate percentage.

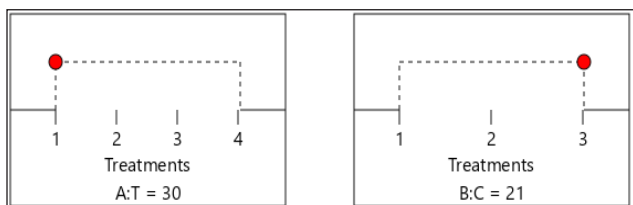


Fig. 3: Optimization parameter of ozone treatment for maximum total carbohydrate percentage

Thus, the result of experiments performed treating wheat seed with ozone makes it clear that ozone holds the total carbohydrate percentage of wheat seed up to its peak point after that if we increase ozone exposure the total carbohydrate starts to decrease. As shown in Fig. 3, optimal parameters given for treating wheat seed with ozone to stimulate total carbohydrate percentage should be exposure time of 30 min and an ozone cycle of 21 days recommended.

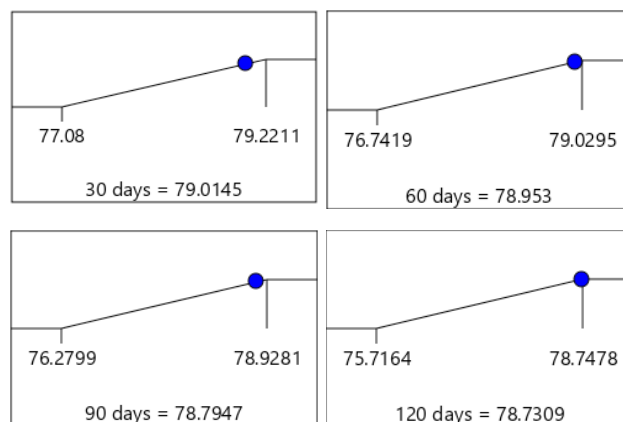


Fig. 4: Ozone total carbohydrate percentage at optimum parameter of ozone treatment after 30 days, 60 days, 90 days and 120 days in wheat bulk storage

At the recommended optimal parameters for treating wheat grains with ozone, continuous observation taken after 30 days, 60 days, 90 days and 120 days. The result shows that exposure time 30 min and ozone cycle at 21 days gives best result of carbohydrate content. As per given in Fig. 4, after 30 days of applied optimum condition of ozone, 79.01% carbohydrate content were found. After 60 days of storage the carbohydrate content start to decrease slowly and found 78.95% carbohydrate in wheat grains. After 90 days of storage, carbohydrate slightly decrease from above month and reach up to 78.79%. At the end of 120 days, the carbohydrate content was 78.73% at desirability of 1 which gives best result in compare to other treatment of ozone exposure.

### CONCLUSION

The experiments conducted in this study demonstrate the effect of ozone on wheat grain carbohydrate content. Ozone has potential for use in storage insect control because it can be generated at site of application, it does not leave toxic residue



after treatment and rapidly control the insect pest infestation. After all of these advantages, ozone gas treatment has negligible effect on carbohydrate content of grain. The total carbohydrate percentage of wheat seeds up to its peak point and then it starts to reduce. The reduction rate also high compared to control treatment. Considering the maximum carbohydrate percentage, ozonation at exposure time of 30 min and ozone cycle of 21 days can be recommended as a non-toxic disinfectant for wheat grains.

## ACKNOWLEDGMENTS

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# Effects of Process Parameters on Rice Based Extruded Snack Food

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## ABSTRACT

Extrusion cooking is a method of transforming raw components into ready-to-eat foods using a high-temperature, short-time shear process. A flour mixture was extruded using a twin-screw extruder. The impact of three independent variables with five varied die temperatures (90, 102, 120, 138, and 150 °C), screw speeds (200, 230, 275, 320, and 350 rpm), and feed moisture content (12, 14, 18, 22, and 24 percent w.b.) on various attributes of extruded items were explored. Extrusion process parameters that were discovered to be the best were 137.83 °C die temperature, 230.40 rpm screw speed and 14.43 percent (w.b) feed moisture content. According to the analysis, the bulk density was 89.37 kg/m<sup>3</sup>, the expansion ratio was 3.16, the water solubility index was 2.77 percent, the water absorption index was 6.34 g/g, the actual protein was 2.49 percent, and the calcium content was 52 mg/100g. Finally, the improved product had a true protein increase of 23.27 percent and a calcium increase of 18.18 percent when compared to the control sample.

## HIGHLIGHTS

- ① Effect of die temperature significantly affect the different properties of extruded snack food.
- ② Effect of feed moisture content significantly affect the different properties of extruded snack food.
- ③ Response surface plot of the different properties were studied and analysed.

**Keywords:** Extrusion cooking, nutrient, die temperature, feed moisture, rice, snack food

Extrusion cooking is a high-temperature, short-duration shear technique for converting raw materials into modified intermediate and finished products (Riaz 2001). Furthermore, extrusion cooking of ready-to-eat items has various advantages over traditional processing methods, including faster processing times, better quality retention, reduced processing costs, and higher flexibility, which allows for a wider range of products (Forsido and Ramaswamy 2011). It has been widely adopted as a popular approach for producing a wide range of food products, from basic enlarged snacks to highly processed meat substitutes.

Cereal grains are the primary basic materials used in the production of extruded snack foods. Because of the high starch content, they provide

good expansion qualities for extruded snack foods. Because of its significant starch content and outstanding expansion capabilities, rice is one of the most commonly used cereal grains for extruded snack food development (Launay and Lisch 1983).

Extruded snacks formulated from cereals are very low in other nutrients such as protein and other functional components (Petrova *et al.* 2015). For increasing the nutritional quality of snacks, new and novel ingredients are being included in the product formulations. So, incorporating of soybean, finger

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millet and mango seed kernel flour was reported to have a positive effect on enrichment in nutrients of product. Soybean is rich source of protein. Finger millet has considered as a rich source of calcium, iron, protein, fibre and other minerals (Salehifar and Shahedi 2007). Mango seed kernel contains most of the essential amino acids. As per record Amino acid composition of mango seed kernel are higher than World Health Organization reference protein for human being (Abdalla *et al.* 2007). Therefore, the focus of this research, to enrich the nutritional value of the extruded product. To achieve that, we used the finger millet, soybean and mango seed kernel as ingredients in rice flour.

## MATERIALS AND METHODS

The extruded product was developed from the composite flour containing rice flour, finger millet flour, soybean flour and mango seed kernel flour.

### Standardization of ratio of flours

The ratio of the different flours was decided based on the preliminary trials that conducted at middle level of independent parameters. Based on sensory evaluation of preliminary trial products, which was got the maximum sensory score was fixed the proportion of composite flour for final experiment. The effect of three independent process variables viz., die temperature, screw speed and feed moisture content on bulk density, expansion ratio, water solubility index, water absorption index, actual protein and calcium were studied with variables. The levels of variables were carefully chosen based on the literature of review available on the extrusion cooking of different food raw materials. Experiments were carried out with five levels of die temperature (90, 102, 120, 138 and 150 °C), screw speed (200, 230, 275, 320 and 350 rpm) and feed moisture content (12, 14, 18, 22 and 24 % w.b.).

### Extruded product preparation

The laboratory co-rotating twin-screw extruder (Basic Technology Pvt. Ltd (BTPL lab model) made, Model EB-10) was used for this experiment. The main drive was provided with 7.5 HP motor (400 V, 3 ph, 50 cycles). Die temperature, screw speed and feed moisture content were set as per desired level with the help of control panel. First, the twin-screw

extruder was run for duration of 30-40 minutes to reach the desired temperatures. The die diameter was selected as 3 mm as recommended by the manufacturer for such type of product. Total 400 g composite flour was prepared for one treatment as per standardize ratio. Sample that contain desired moisture content was fed in to feed hopper at the 14 rpm feeder speed and 230 rpm cutter speed. The extruded product was coming out in approx. 20 sec after the feeding the flour. The extruded product was dried using laboratory tray drier at 60 °C temperature for 15 minute for the stabilization of moisture or to remove extra moisture from the product.



Plate 1: Laboratory co-rotating twin-screw extruder

### Properties of extruded product

Different essential properties of extruded product such as bulk density, expansion ratio, water solubility index, water absorption index, actual protein (true protein) and calcium were determined as described in the subsequent section here under.

#### Bulk density

The bulk density of dried extruded product was calculated by determining the volume of extruded product by filling a container of known volume and noting the sample weight (Mohsenin NN 1986).

$$\text{Bulk density, } \left( \frac{\text{kg}}{\text{m}^3} \right) = \frac{\text{Mass of Extrudate sample}}{\text{Volume of container}}$$

#### Expansion ratio

The ratio of diameter of extruded product and the diameter of die was used to express the expansion of extruded product (Fan *et al.* 1996). The diameter of extruded product was determined as the mean





of 10 random measurements made with a Venire calliper having least count 0.1 mm.

$$\text{Expansion ratio} = \frac{\text{Extrudate diameter (mm)}}{\text{Die diameter (mm)}}$$

### Water Solubility Index and Water Absorption Index

Water Solubility Index (WSI) and Water Absorption Index (WAI) were determined by the method described by Anderson (1982a).

Water Solubility Index (%) =

$$\frac{\text{Mass of dissolved solids in supernatant (g)}}{\text{Mass of dry solids (g)}} \times 100$$

$$\text{Water Absorption Index} \left( \frac{\text{g}}{\text{g}} \right) = \frac{\text{Mass of sediment (g)}}{\text{Mass of dry solids (g)}}$$

### Actual protein (True protein) and calcium

Actual protein content of extruded product was determined by Folin-Lowry method (Lowry *et al.* 1951). Calcium content of extruded product was determined by Versenate EDTA method (Carr and Frank 1956).

### Experimental design and statistical analysis

The experimental design was done using Central Composite Rotatable Design (CCRD) of Response Surface Methodology (RSM) that gave minimum number of experiments. The statistical analysis of the experimental data was carried out to observe the effect of selected process parameters on the various responses. The obtained data were subjected to analyze for graphical representation, analysis of variance (ANOVA) and multiple regression using the software package design Expert 10 (Anderson and Whitcomb 2005). The three-dimensional (3D) response surface plots were generated by keeping one variable constant at the centre point and varying the other two variables within the experimental range. The effect of regression coefficients of individual linear, quadratic and interaction terms were determined from the ANOVA tables. The significance of all terms in the polynomial equation was judged statistically by computing the F-value at a probability (P) value of 0.001, 0.01 or 0.05.

### Validity test

The optimum condition obtained through statistical analysis was verified by conducting the experiment in triplicates. The average experimental value of different response variables were used to check the validity and adequacy of the predicted models.

## RESULTS AND DISCUSSION

Depending on sensory evaluation the feed composition were fixed as 75 % rice, 5 % finger millet, 7.5 % soybean and 12.5 % mango seed kernel flour. The final experiments were conducted with same feed composition at different process parameters viz. die temperature, feed moisture content and screw speed. Results of bulk density, expansion ratio, true protein, calcium and sensory were tabulated in Table 1 and Analysis of variance (ANOVA) and regression coefficients for response surface were tabulated in Table 2.

### Effects of process parameters on bulk density

Bulk density of extruded product was ranged from 91.12 to 363.34 kg/m<sup>3</sup> presented in Table 1. Fig. 1a indicated the decrease in bulk density as the die temperature increase up to maximum level. The bulk density increase with an increase in screw speed up to certain level then further increase in screw speed bulk density decreased. Fig. 1b indicated that there was increase in bulk density with an increase in feed moisture content up to maximum level. Shruthi *et al.* (2017) reported the increasing in level of die temperature resulted in decreases bulk density of extruded product. Baik *et al.* (2004) reported similar trends of bulk density of extruded product increase with increase in feed moisture content because higher temperature provided a higher potential energy for flesh-off of super-heated water from extruded product as they left the die. Increase in feed moisture content during extrusion may reduce the elasticity of the dough through plasticization of the melt, therefore increasing the density of extruded product.

It can be seen from the table 2, feed moisture content showed positive linear effect which there were significant at p<0.001 but die temperature showed negative linear effect on bulk density which was significant at p<0.001. Whilst, the interaction effect of die temperature and feed moisture content was

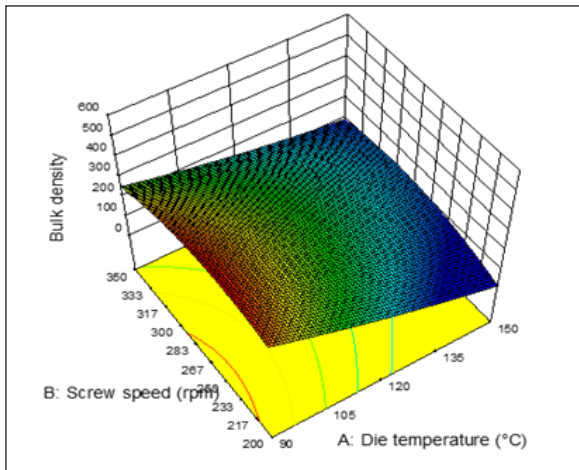
**Table 1:** Effect of extrusion cooking variables on different characteristics of extruded product

Std. run	Independent process variables					Responses			
	Die temperature (°C)	Screw speed (rpm)	Feed moisture content (%)	Bulk density (kg/m <sup>3</sup> )	Expansion ratio	WSI (%)	WAI (g/g)	True protein (%)	Calcium (mg/100g)
14	120	275	24	315.26	2.06	2.27	5.09	2.63	44
17	120	275	18	239.36	2.8	1.59	5.79	2.72	44
12	120	350	18	166.84	2.88	1.9	5.92	2.56	44
13	120	275	12	113.1	3.21	3.5	6.27	2.68	48
9	90	275	18	363.34	1.98	5.24	5.2	3.23	52
20	120	275	18	242.44	2.58	2.68	5.72	2.81	32
15	120	275	18	240.38	2.53	2.37	5.79	2.65	44
5	102	230	22	360.66	2.09	2.7	4.72	3.03	56
4	138	320	14	95.46	3.25	2.03	6.35	2.35	44
16	120	275	18	241.12	2.59	2.16	5.49	2.69	40
19	120	275	18	243.56	2.54	2.88	6.02	2.79	40
7	102	320	22	341.42	1.8	3.19	5.14	2.98	48
11	120	200	18	161.92	2.43	2.43	5.86	2.78	40
1	102	230	14	212.18	2.68	3.6	5.88	3.01	60
18	120	275	18	239.36	2.14	1.96	5.93	2.7	40
3	102	320	14	171.34	2.84	2.36	6.17	2.92	44
2	138	230	14	91.12	3.31	2.71	6.36	2.59	60
8	138	320	22	182.48	2.5	1.17	6.19	2.2	60
6	138	230	22	133.04	3.17	1.21	6.04	2.25	36
10	150	275	18	123.64	2.93	1.16	6.29	1.78	52
Control	120	275	18	133.44	3.4	6.33	9.36	2.02	44

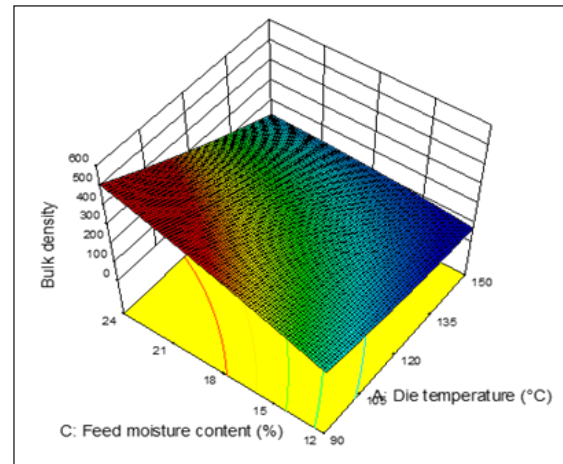
**Table 2:** Analysis of variance (ANOVA) and regression coefficients for response surface quadratic model of different characteristics of extruded product

Source	Bulk density	Expansion ratio	WSI	WAI	True Protein	Calcium
Intercept	223.32	2.53	2.29	5.79	2.72	39.87
<b>Linear terms</b>						
(X <sub>1</sub> )	-72.24***	0.32***	-0.85***	0.36***	-0.37***	-0.59
(X <sub>2</sub> )	0.14	-7.556E-003	-0.17	0.070	-0.059*	-0.68
(X <sub>3</sub> )	57.66***	-0.33***	-0.33	-0.34***	-0.036	-1.08
<b>Interaction terms</b>						
(X <sub>1</sub> X <sub>2</sub> )	14.23	-0.075	2.500E-003	-0.071	-0.019	4.00
(X <sub>1</sub> X <sub>3</sub> )	-23.70*	0.093	-0.29	0.21**	-0.071*	-1.00
(X <sub>2</sub> X <sub>3</sub> )	8.34	-0.13	0.30	0.036	0.029	6.00*
<b>Quadratic terms</b>						
(X <sub>1</sub> <sup>2</sup> )	5.55	-1.152E-003	0.24	2.174E-003	-0.065*	5.07**
(X <sub>2</sub> <sup>2</sup> )	-22.42***	0.070	-0.12	0.053	-6.671E-003	1.54
(X <sub>3</sub> <sup>2</sup> )	-4.82	0.062	0.13	-0.021	-0.012	2.95
<b>Indicators for model fitting</b>						
R <sup>2</sup>	0.9541	0.8686	0.8239	0.9442	0.9609	0.7474
Adj-R <sup>2</sup>	0.9127	0.7504	0.6653	0.8939	0.9258	0.5200
Pred-R <sup>2</sup>	0.9174	0.3813	0.0425	0.8288	0.7576	-0.6000
Adeq Precision	15.259	9.016	8.782	15.537	19.282	5.921
F-value	23.08	7.35	5.20	18.79	27.34	3.29
Lack of fit	NS	NS	NS	NS	NS	NS
C.V. %	12.07	8.48	22.67	2.60	3.38	12.01

X<sub>1</sub>= Die temperature, X<sub>2</sub>= Screw speed, X<sub>3</sub>= Feed moisture content, \*\*\*Significant at <0.001, \*\*Significant at p<0.01, \*Significant at p<0.05, NS = Non-significant.

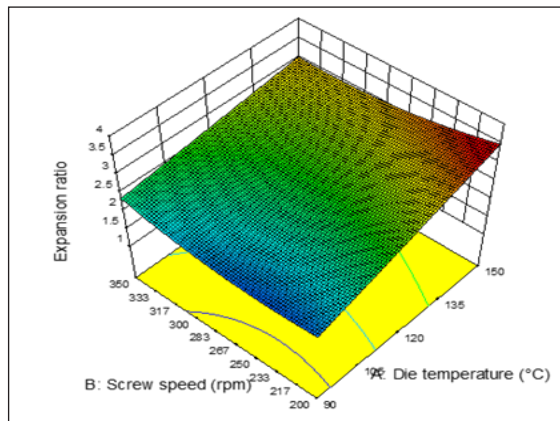


(a)

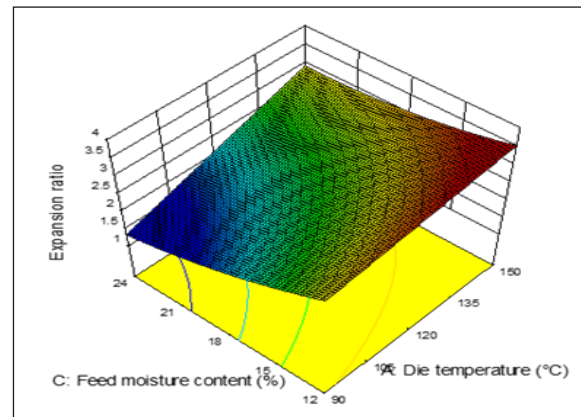


(b)

**Fig. 1:** Response surface plot for bulk density of extruded products as a function of die temperature, screw speed and feed moisture content



(a)



(b)

**Fig. 2:** Response surface plot for expansion ratio of extruded products as a function of die temperature, screw speed and feed moisture content

significantly negative at  $p < 0.05$  and the quadratic effect of screw speed was negatively significant at  $p < 0.001$  on bulk density.

The calculated F-value for bulk density (23.08) was significant at  $p < 0.001$ . At the same time, it possessed non-significant lack of fit ( $p > 0.05$ ). These values indicated that the model for bulk density was fitted and reliable. The  $R^2$  value and Adj- $R^2$  value higher than the 0.8, indicating the adequacy, good fit and high significance of the model. The Pred- $R^2$  was in reasonable agreement with the Adj- $R^2$ . The high Adeq. Precision value ( $> 4$ ) again supported the significance of the model. The small value of coefficient of variation (12.07 %) explained that the experimental results were precise and reliable.

### Effects of process parameters on expansion ratio

Expansion ratio of extruded product was ranged from 1.8 to 3.31. Fig. 2a indicated the increase in expansion ratio as the die temperature increases and decrease in expansion ratio as screw speed increases up to certain level and further increase in screw speed expansion ratio increase. Fig. 2b indicated that there was increased in expansion ratio with an increase in die temperature up to maximum level and decrease in feed moisture content up to minimum level.

Gat and Ananthanarayan (2015) observed that expansion of extruded product decrease with increase in feed moisture content and increase in

die temperature lead to slight decrease expansion value. Stojceska *et al.* (2009) also observed similar findings and reported expansion ratio increase with increase in screw speed. High temperature results in larger starch gelatinization. It was explained that melt viscosity decrease with increase temperature and the reduced viscosity effect would favour the bubble growth during extrusion.

Die temperature and feed moisture content showed positive and negative linear effect respectively which were significant at  $p < 0.001$  but screw speed showed negative linear effect on expansion ratio which was non-significant at  $p > 0.05$ . Whilst, the interaction effect and quadratic effect on expansions ratio were non-significant. The calculated F-value of 7.35 was significant at  $p < 0.01$ . At the same time, it possessed non-significant lack of fit ( $p > 0.05$ ). The  $R^2$  value and Adj- $R^2$  value indicating the adequacy, good fit and high significance of the model. The high Adeq Precision value ( $>4$ ) again supported the significance of the model. Coefficient of variation of 8.48 % explained that the experimental results were precise and reliable represented in Table 2.

### Effects of process parameters on Water Solubility Index (WSI)

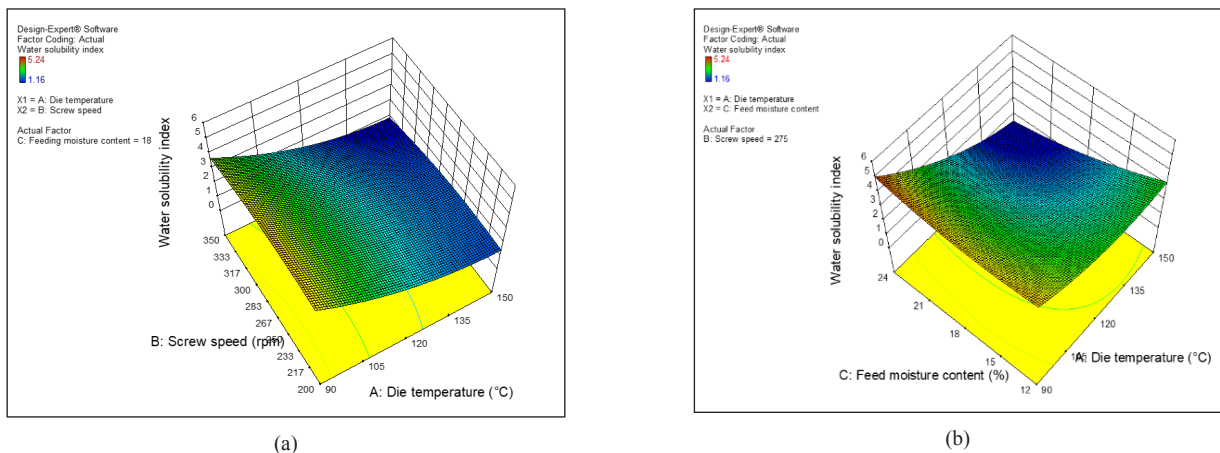
Water solubility index of extruded product was ranged from 1.16 to 5.24 %. Fig. 3a indicated decrease in water solubility index as the die temperature increase and increase with increase in screw speed. Fig. 3b indicated increase in water solubility index with a decrease in die temperature and increase in feed moisture content. Gat and Ananthanarayan

(2015) observed that water solubility index of extruded product decrease with increase in feed moisture content. It may be observed to reduction in lateral expansion due to plasticization of melt.

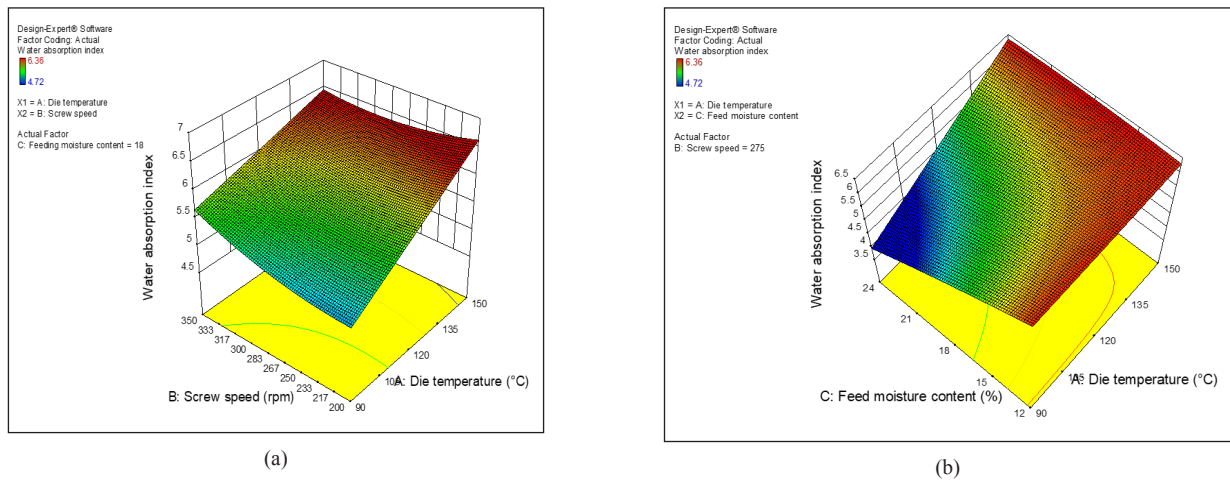
The regression analysis and ANOVA results for the water solubility index of extruded product are shown in the Table 2. It can be seen from the table, that die temperature showed negative linear effect which was significant at  $p < 0.001$  but screw speed and feed moisture content showed negative linear effect on water solubility index which was non-significant at  $p > 0.05$ . Whilst, the interaction and quadratic effect were non-significant on water solubility index. The calculated F-value for bulk density (5.20) was significant at  $p < 0.01$ . At the same time, it possessed non-significant lack of fit ( $p > 0.05$ ). These values indicated that the model for water solubility index was fitted and reliable. The  $R^2$  value and Adj- $R^2$  value for the water solubility index were 0.8239 and 0.6653, respectively, indicating the adequacy, good fit and high significance of the model. The high Adeq. Precision value ( $>4$ ) again supported the significance of the model for bulk density. The value of coefficient of variation (22.67%) for water solubility index explained that the experimental results were precise and reliable.

### Effect of process parameters on water absorption index (WAI)

Water absorption index of extruded product was ranged from 4.72 to 6.36 g/g. Fig. 4a indicated increase in water absorption index as the die temperature increase and decrease with screw



**Fig. 3:** Response surface plot for Water Solubility Index of extruded products as a function of die temperature, screw speed and feed moisture content



**Fig. 4:** Response surface plot for Water Absorption Index of extruded products as a function of die temperature, screw speed and feed moisture content

speed. Fig. 4b indicated increase in water absorption index with an increase in die temperature and decrease in moisture content.

Alam *et al.* (2015) observed WAI decrease slightly with increasing screw speed to a certain level and then increase, whereas an increasing trend was observed with increasing die temperature. Results have been found by Stojceska *et al.* (2010) that WAI increase with increasing die temperature. Water absorption index decreased with increase in moisture content, which may be attributed to the reduction of elasticity of dough through plasticization of melt at higher moisture content and increase in increase in temperature probably due to increased dextrinization of at higher temperature.

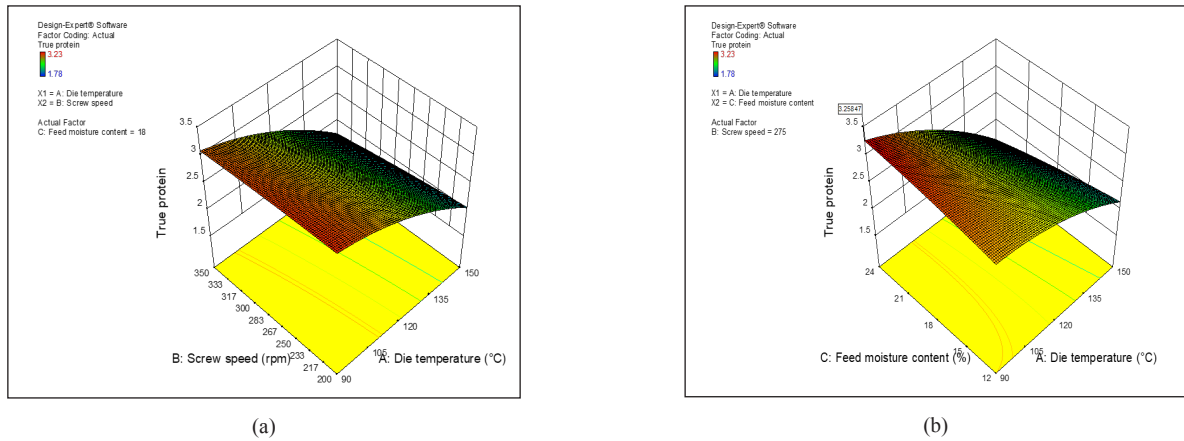
The regression analysis and ANOVA results for the water absorption index of extruded product are shown in the Table 2. It can be seen from the table, that die temperature and feed moisture content showed positive and negative linear effect, respectively, which were significant at  $p < 0.001$  but screw speed showed positive linear effect on water absorption index which was non-significant at  $p > 0.05$ . Whilst, the interaction effect of die temperature and feed moisture content showed positively significant at  $p < 0.01$  and the quadratic effect on water absorption index was non-significant. F-value for water absorption index (18.79) was significant at  $p < 0.001$ . At the same time, it possessed non-significant lack of fit ( $p > 0.05$ ). These values indicated that the model for water absorption index was fitted and reliable. The  $R^2$  value and Adj- $R^2$  value for the water absorption index were

0.9442 and 0.8939, respectively, which were higher than the 0.8, indicating the adequacy, good fit and high significance of the model. The Pred- $R^2$  (0.8288) was in reasonable agreement with the Adj- $R^2$ . The high Adeq. Precision value ( $>4$ ) again supported the significance of the model for water absorption index. The small value of coefficient of variation (2.60 %) for water absorption index explained that the experimental results were precise and reliable.

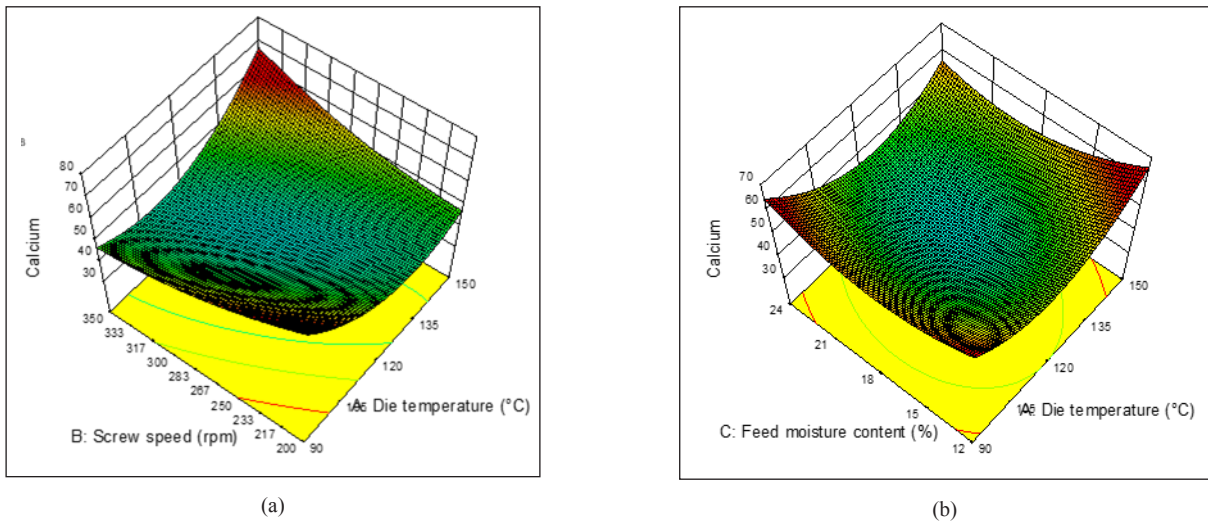
### Effect of process parameters on true protein

True protein of extruded product was ranged from 1.78 to 3.23 %. Fig. 5a indicated the decrease in true protein as the increase in die temperature and screw speed because increase in temperature protein denaturation occur. Fig. 5b indicated that true protein decrease with increase in feed moisture content.

It can be seen from the Table 2, the linear effect of die temperature and screw speed negatively significant at  $p < 0.001$  and  $p < 0.05$ , respectively. The interaction effect of die temperature and feed moisture content was significantly negative at  $p < 0.05$  and the quadratic effect of die temperature was positively significant at  $p < 0.05$  on true protein. F-value for true protein (27.34) was significant at  $p < 0.001$ . At the same time, it possessed non-significant lack of fit ( $p > 0.05$ ). The  $R^2$  value and Adj- $R^2$  value indicating the adequacy, good fit and high significance of the model. The high Adeq. Precision value ( $>4$ ) again supported the significance of the model. The value of coefficient of variation (3.38 %) explained that the experimental results were precise and reliable.



**Fig. 5:** Response surface plot for true protein of extruded products as a function of die temperature, screw speed and feed moisture content



**Fig. 6:** Response surface plot for calcium of extruded products as a function of die temperature, screw speed and feed moisture content

### Effect of process parameters on Calcium

Calcium of extruded product was ranged from 32 to 60 mg/100g. Fig. 6a which indicated the decrease in calcium as the die temperature was increased up to 119 °C and screw speed up to 287 rpm. The calcium at this combination was proposed to be decreased up to 39.79 mg/100g. With further increase in screw speed and die temperature, the calcium of extruded product increase. Fig. 6b indicated decrease in calcium with an increase in die temperature up to 121 °C and feed moisture content up to 19 %. At this combination, the calcium of extruded was predicted up to 39.75 mg/100g with further increase in die temperature and feed moisture content, the calcium increases.

It can be seen from the table 2, the interaction effect of screw speed and feed moisture content was significantly positive at  $p < 0.05$  and the quadratic effect of die temperature was positively significant at  $p < 0.01$  on calcium. Other effect of parameter was non-significant on calcium. The calculated F-value for calcium (3.29) was significant at  $p < 0.05$ . At the same time, it possessed non-significant lack of fit ( $p > 0.05$ ). The  $R^2$  value and Adj- $R^2$  value indicating the adequacy, good fit and high significance of the model. The high Adeq. Precision value ( $> 4$ ) again supported the significance of the model. The small value of coefficient of variation (12.01 %) for calcium explained that the experimental results were precise and reliable.



## Optimization and validation of process variables

The optimum condition determined using Design Expert software version 10 (State-Ease Inc., Minneapolis, MN, USA). The main criteria applied for constraints optimization in the study were bulk density is minimum, expansion ratio, WSI, WAI, actual protein and calcium were maximum. Optimum treatment conditions were found to be, 137.83 °C die temperature, 230.40 rpm screw speed and 14.43 % (w.b) feed moisture content. The analysis showed that at this combination, it would be possible to produce an extruded product with a bulk density of 89.37 kg/m<sup>3</sup>, expansion ratio of 3.16, water solubility index of 2.77 %, water absorption index of 6.34 g/g, actual protein of 2.49 % and calcium of 52 mg/100g with a desirability of 0.815.

## CONCLUSION

Composite flour was prepared from proportion of 75 % rice flour, 5 % finger millet flour, 7.5 % soybean flour and 12.5 % mango seed kernel flour. It can be suggested for enhance the nutritional value of extruded snack obtained by 137.83 ° die temperature, 230.40 rpm and screw speed and 14.43 (% w.b.) feed moisture content which gave the higher values of nutrients. Die temperature and feed moisture content both process parameters can significantly affect on different properties of extrude snack food. Screw speed cannot be significantly affect on different properties of extruded snack food. In control sample, true protein and calcium were found to be 2.02%, 44 mg/100g, respectively (Table 1). Hence, there was an increase in true protein of 23.27 % and calcium of 18.18 % over control sample.

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# Monitoring and Regulating Climatic Condition of Polyhouse for Successful Off-season Grafting of Citrus Fruits Using Internet of Things Platform

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## ABSTRACT

Citrus fruit (mandarin, sweet orange and acid lime) is one of the most cultivated and consumed fruits in Nepal. The demand for citrus plants is increasing every year. However, due to the prevailing climatic conditions the citrus plants are mostly grafted once a year during November to January in Nepal. The average ambient temperature during this period is around 5-22°C. Previous research has shown that the grafting success rate during this window has been found to be above 90% when using small plastic tunnels. This grafting period has been a bottleneck to meet the demand of the citrus plants. The other problem is; these young plants are sent for transplantation in June-July of the same year which are merely 5-6 months young. This could increase the chances of mortality rate during transplantation. To address the problem of demand and supply as well as to extend the grafting window period an investigation with robust climate monitoring and climate regulating system is proposed for internal climate management inside a poly house of National Citrus Research Program, Dhankuta, Nepal to carry out the experiment on off season grafting of citrus fruit. An internet of things (IoT) based design and automation is implemented in a polyhouse for off season grafting, which has not been practiced so far in Nepal. The sensors and actuators are equipped and placed appropriately inside the polyhouse. The IoT platform is designed and deployed to acquire the sensor data in real-time that helps to visualize, monitor and regulate the internal climate inside the polyhouse. This system not only limits the potential to graft citrus plants but also can be extended to other fruits like avocado, walnut, apple, pear, and peach, which grafting success rate is low at present when carried out in an open field conditions.

## HIGHLIGHTS

- ① The study of this research could help to perform off-season grafting inside a polyhouse by regulating the internal microclimate using the internet of things.
- ② This technology further could be extended to other fruit varieties for grafting.

**Keywords:** Citrus Grafting, Climate monitoring and regulation, Sensor automation, Real time data acquisition, Internet of Things

Nepal is a south Asian country with diverse climatic and geographical terrain. The topographic landscape of Nepal is mainly divided into three regions namely, the Mountain region, Mid-hill, and

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the Terai region. *Citrus* (mandarin, sweet orange and acid lime) is one of the most cultivated fruits in the mid-hill region which lies 700-1400 m above the sea level. The climatic conditions in these regions are suitable for cultivation. The *Terai* region ranges from 65- 300 m above sea level and some varieties of citrus fruits are cultivated in this region. The farmers in both regions are attracted towards cultivating citrus fruit as it is largely consumed and has a good market price (NCRP, 2018). It has been found from the sources that the demand for the citrus fruits and saplings are increasing annually. To cater the need for the demand of citrus saplings, grafting operations are done. However, the grafting is performed only once in a year in government research center and other private nurseries in an open field condition during winter season (November- January) as the climatic conditions are favorable during this period. This grafting window has limited the number of graftage, which are not able to meet the escalating demand of the farmers. Taking this advantage, it has been reported that some private nursery houses are selling low quality saplings to the farmers. The off-season grafting in natural condition is not in practice in Nepal since the grafting success rate is very poor (Bhandari and Regmi 2021). Various research on grafting is carried out over the years to improve the grafting success rate however the off-season grafting is still under practice in Nepal. In other parts of the world citrus fruits are grafted/budded in early spring and sold another year in June-July. This grafting operation is done inside a climatic controlled structure called high-tech nursery house. The grafted plant under such conditions is one-year-old when taken for transplantation and has less mortality rate. Nepal has not utilized such technology and structures for citrus fruit grafting so far. This paper proposes the techniques for off season grafting of citrus fruit using internet of things and automation in a polyhouse to achieve a controlled environment grafting.

## MATERIALS AND METHODS

The field experiment of grafting operations is conducted at National Citrus Research Program (NCRP), Dhankuta, Nepal. NCRP, which conducts special research on citrus fruits is established under the Nepal Agricultural Research Council (NARC) (Bhandari and Regmi 2021).

## On season regular grafting

The regular grafting operation is performed in an open field during the winter season i.e., Nov-Jan (On season grafting). Samples size, grafting methodology, root stock, scion, grafting dates are noted. The temperature, humidity is continuously monitored and recorded. After the grafting operation the graftage is kept inside a low plastic (200 micron) tunnel as shown in Fig. 1(a) whose dimensions are 75 cm height and 50 cm wide respectively. The saplings are regularly irrigated to raise the humidity level to 85-95% each day. The grafted plants are kept inside the tunnel for six months. It has been observed that the graftage starts to sprout in around 21 days and grows into a self-sustaining plant. Due to various reasons some of the grafting may not be successful. The grafting success rate is calculated using the relation below as suggested by chalise *et al.* (Chalise *et al.* 2013).

$$\text{Graft Success \%} = \frac{\text{Number of sprouted grafts}}{\text{Total number of grafts}} \times 100$$

Similarly, the mortality rate can be estimated as;

$$\text{Mortality (\%)} = \frac{\text{Number of dead grafts after sprouting}}{\text{Total number of sprouted grafts}} \times 100$$

The success rate of graftage along with the temperature and humidity are analyzed. These parameters are taken as a reference while conducting the off-season grafting.

As shown in the Fig. 1 (a) the temperature and humidity inside the low tunnel is recorded periodically using an IoT device. The variation in temperature, humidity is stored and analyzed over the period of time. The on season grafting success rate is usually found to be more than 90% (Bhandari *et al.*). The experimental result has shown that the range of the temperature and humidity suitable for the success of the grafting is in the range of 21-29° C and 85-95 % respectively (Bhandari *et al.* and Chalise *et al.* 2013). This data is used to correlate with the microclimate that is required inside the poly house while performing off-season grafting. The off-season grafting is conducted during July-August as shown in table 1.

An experiment setup has been installed to extend the off season grafting window during July- August

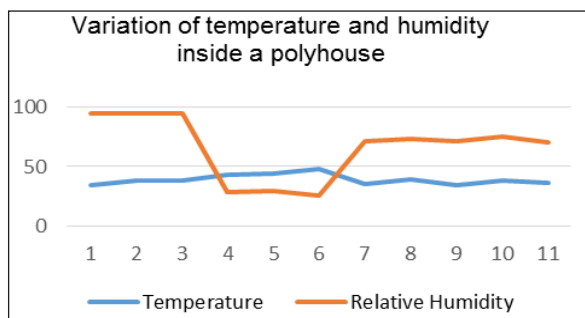


**Fig. 1:** (a) IoT device used in season grafting to store temperature, humidity (b) Graftage plants inside the polyhouse (c) off season graftage inside the polyhouse in controlled environment

**Table 1:** Off-season grafting record

Grafting date	Root stock	Scion	Grafting method	Graftage number	Replication
July 1				10	3
July 15				10	3
July 30				10	3
Aug 1	Trifoliolate	Acid Lime (Sunkagati-1)	Veneer	10	3
Aug 15				10	3
Aug 30				10	3

at an interval of 15 days. For this a polyhouse of NCRP is utilized. The dimension of the polyhouse is 160 m<sup>2</sup> which is 20 m long, 8 m wide and 9 m tall. To automate and regulate the internal micro-climate of the polyhouse; cooling pad, heating system, exhaust fan and foggers are installed. The polyhouse is equipped with sensors and actuators to monitor and regulate the internal microclimate. The system design is based on the internet of things platform.



**Fig. 2:** Temperature, Humidity variation inside a polyhouse during May-June

The ambient air temperature in the experiment site during June-August ranges from min 15 to max 30° C which is an off season grafting window. The temperature and humidity inside the polyhouse is acquired using sensors which are also sent to the cloud server using Internet of Things. It is found that the temperature inside the polyhouse reaches as

high as 50° C and relative humidity falls below 30% as shown in Fig. 2. The challenge is to regulate the internal microclimate to suit the best range of the temperature and humidity as mentioned above. This is achieved by operating the exhaust fan, cooling pad and fogger system using Internet of Things.

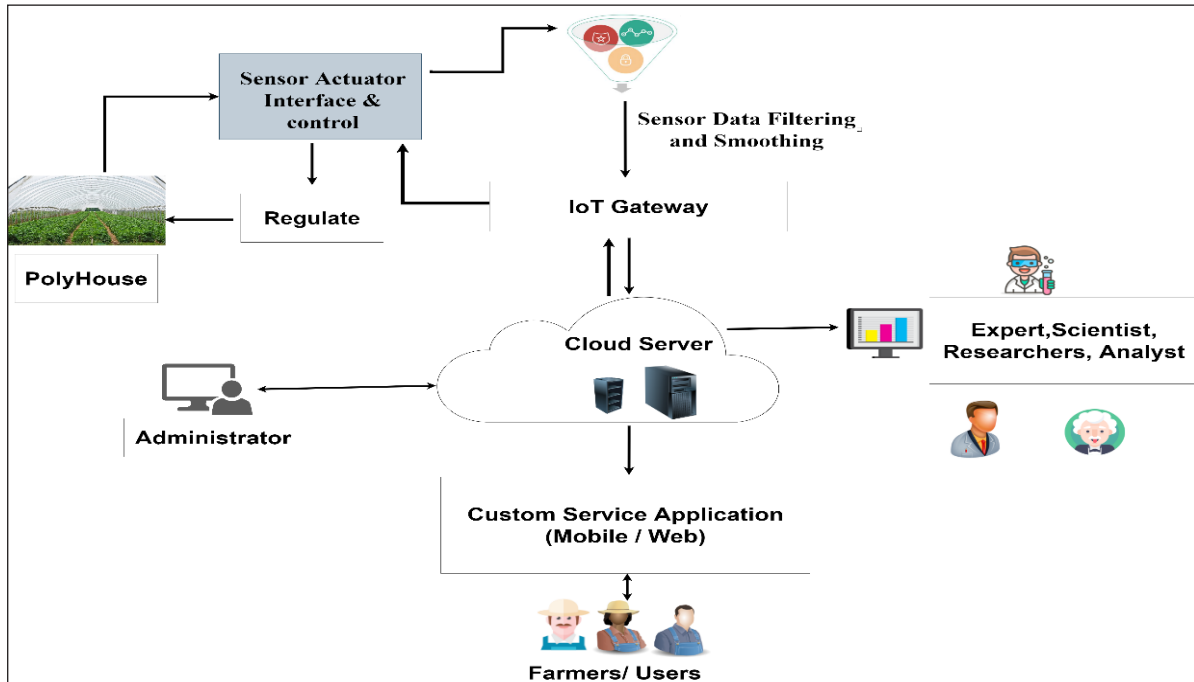
Further, the sapling height, diameter, leaf area, number of leaves, success rate etc. are noted in the first, third and sixth months after the grafting as shown in table 2.

### Internet of Things, System Design and Architecture

The Internet of Things (IoT) is an emerging technology which uses electronic devices and sensors that communicate over the internet in order to make human lives easy (Kumar *et al.* 2019). IoT devices are capable of collecting the data and exchanging the information through the internet in real time. IoT has been recently used in many domains and applications. This has helped to save cost, labor and time effectively. The concept of smart city, smart home, and smart agriculture is driven by the Internet of Things (Nizetic, Sandro *et al.* 2020). The use of IoT in various agriculture sectors has been significantly growing (Andrés Villa-Henriksen *et al.* 2020). It is believed that the

**Table 2:** Sample inspection of grafting

Grafting Date	Rep	Sample no	Sapling Height(cm)	Increased in diameter above the union	Graft Success	Graft Unsuccessful	No of Leaves	leaf Area (Cm <sup>2</sup> )	No of primary branches/sapling
July 1	1	1-5			No	No			
	2	1-5							
	3	1-5							
July 15	1	1-5							
	2	1-5							
	3	1-5							

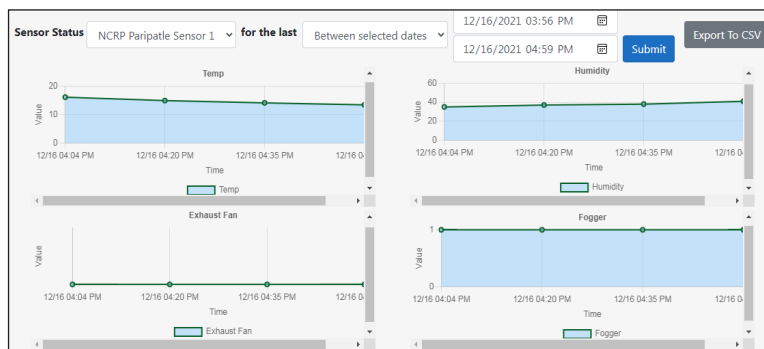


**Fig. 3:** System Architecture of Internet of Things

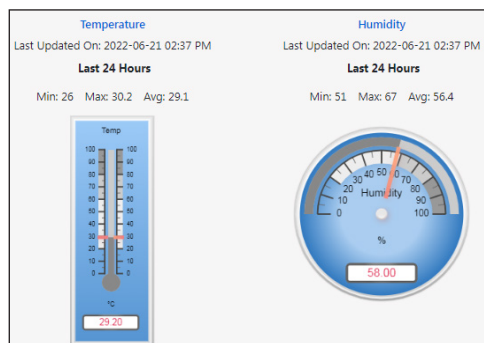
use of IoT devices in the agriculture industry is growing 20% annually (Muangprathub *et al.* 2019). Traditional method of cultivation is not able to meet the ever rising demand for agricultural products. Innovative technologies and methodologies need to be implemented to meet the increasing demand for food supply (Singh and Singh 2017). IoT is one of the promising technologies for smart agriculture. A robust and reliable Internet of Things based system is designed for the proposed off-season grafting that supports multifunctional and scalable architecture. The system architecture is shown in Fig. (3) which is designed based on the Internet of Things. This system architecture is designed to acquire the sensor data, visualize and regulate the appliances inside the polyhouse. Parameters like temperature, humidity inside the polyhouse are acquired using the sensors. A microcontroller is

interfaced with the sensor to filter and provide the sensor data at regular intervals. An IoT gateway then sends this data to the cloud server. The cloud server can be accessed by the users, experts, researchers using mobile and web applications to visualize the sensor data. The user can also issue the external command using this application to regulate the internal microclimate of the polyhouse. The sensor data is stored in the cloud server and can be retrieved for data analytics purposes. Users are provided with the login credentials that provide access to the mobile and web application. The major features include dashboard for visualization, switch control for regulation and data export for data analytics.

The temperature humidity inside the polyhouse is regulated using the IoT platform to the desired range as shown in table 3. The designed IoT system



(a)



(b)

Fig. 4: (a) Time Series Sensor Data (b) Dashboard

is able to show the time series data as well as a dashboard for visualization as shown in Fig. (4).

Table 3: Sample of Regulated temperature, humidity inside the polyhouse using IoT

Date	Time	Temperature (°C)	Relative Humidity %
2022/5/13/	12:07	32.8	68
2022/5/13/	12:15	30.8	74
2022/5/13/	12:21	30.8	74
2022/5/13/	12:27	29.4	78
2022/5/13/	12:33	28.5	80
2022/5/13/	12:40	27.6	82
2022/5/13/	12:46	27.1	85
2022/5/13/	12:52	26.7	86
2022/5/13/	12:58	26.7	89
2022/5/13/	13:05	28.9	83
2022/5/13/	13:11	28.9	84

## RESULTS AND DISCUSSION

The off-season grafting of citrus fruits using informed decision from IoT and automation in a Polyhouse is a relatively new concept in Nepal. Natural open field grafting windows are not able to meet the demand of the citrus saplings. The research done in the past has suggested that temperature and humidity are vital parameters that impact the success rate of the grafting. The data obtained during the seasonal grafting whose grafting success rate is higher is taken as a reference for conducting off season grafting in a controlled environment in the coming season. The internal microclimate inside the polyhouse is maintained to the desired range using exhaust fan, cooling pad, fogger, heating system etc. through internet of things. The

cloud server is interfaced using mobile and web applications to visualize and also to control the devices from anywhere anytime. An IoT platform is designed to regulate the internal microclimate of the polyhouse for off season grafting. The grafting operation will be performed during July- August 2022, using the IoT system. The corresponding data entry is done as shown in table 2. The overall success rate of the graftage is then calculated. The success of this experiment will lead to extending the grafting window period to conduct off-season grafting not only for citrus fruits but also to other varieties like avocado, walnut, apple, pear and peach which are still under research.

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# Climate Resilient Practices Adopted in Flood and Drought Prone Areas of Siwan District, Bihar

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## ABSTRACT

Climate Resilient Agriculture (CRA) is need of hour in many parts of the World when the variability of climates is concerned. In this regard, the CRA project funded by Government of Bihar, India is a state wide project which addresses the climate change scenario further its influence on cropping pattern and promoting the strategies to be made to achieve sustainable production. Three seasons with different cropping system were entailed during the start of the project from *Rabi* 2020-21, Summer-2021 and *Kharif*-2021. The *Rabi* sown zero till wheat in CRA plots recorded higher yield of 32.80 q ha<sup>-1</sup> with increase of yield of 23.02 per cent over farmers' practise (Control). Similar trend of improvement in yield and net returns were observed in CRA experimental plots compared to control of respective climate resilient practises during *Rabi*-2021. Summer sown *moong* bean also followed similar trend of increase in yield of 11.00 q ha<sup>-1</sup> over the control (7.22 q ha<sup>-1</sup>) treatment. During *Kharif*-2021, Paddy sown with DSR practises recorded higher yield (41.00 q ha<sup>-1</sup>) compared to farmers' practise. Nutrient expert (LCC) based fertilizer application and Community irrigation plots recorded similar trend of increase in yield over the control. The results suggested that an adoption of CRA practises improved productivity and also saved time and expenditure towards crop production.

## HIGHLIGHTS

- ① Climate Resilient practises in Siwan district reduced the risk of climate vagaries on crop growth and development.
- ① Increased yield in farmers' field.
- ① Better water management.
- ① Community irrigation reduced crop failure due to moisture stress in the areas of research.
- ① Climate Resilient practises increased farmers' income.

**Keywords:** Climate Resilient Agriculture, Zero-tillage, DSR, LCC, Raised bed Planting, Community Irrigation

Water stress accompanied with climate change are commonly occurring in Bihar. As per National Planning Commission, Siwan district falls under Middle Indo-Gangetic Plain soils of India. It extends between north latitude 25° 22' and 26° 27' and East Longitude 84° 2' and 84° 46'. Monsoon sets sometimes in the third week of June and it lasts till the end of September. The average annual rainfall in Siwan district is 1029.03 mm. The maximum rainfall

in the district comes from South West monsoon with a little about 10% spread over the summer and winter. There is a large variation in the rainfall

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over year to year. Rainfall increases from Southwest to north-east parts of the district (Central Ground water Board 2013). The climate of the district is sub-tropical to sub-humid in nature. The district experiences severe cold during winter whereas on the other hand in summer it is very hot. The summer starts from mid of March and it continues up to mid of June after that monsoon starts and it goes up to mid of October. The nights are generally hot from the end of May till the first break of monsoon. The climate is generally hot and dry, the winter temperature ranges from 16°C to as low as 4°C whereas during the summer the mercury shoots to 46°C. During rainy season it becomes cooler and temperature drops to 35°C to 25°C. Considering the different conditions like texture, climate and pedo-genetic situation, the Siwan district is characterised; by a wide variety of soils which can be broadly grouped into *Entisols* and *Inceptisols* as under as per the U.S. Survey staff (1975). Heat waves, drought and floods are occurring during the cropping season and are ultimately reducing the crop yield over the district. Keeping these points in view, climate resilient practises such as DSR, raised bed planting of maize and arhar (Red gram) and community irrigation practises were entailed in the research plots and farmers’ field.

**MATERIALS AND METHODS**

Climate Resilient Agriculture (CRA) project was started during *Rabi*, 2020 officially inaugurated by Hon’ble Chief Minister of Bihar on 14<sup>th</sup> December, 2020. Progressive farmers’ field were selected as research plot and existing farmer’s practise was considered as the respective experimental control under different climate resilient practises and season. Project location selected and their respective blocks of the Siwan district are presented in Table 1.

**Table 1:** Location selected, number of farmers and area of climate resilient agriculture practised during different cropping season

Sl. No.	Project location	Block
1	Saidpura	Goriakothe
2	Kaladumra	Goriakothe
3	Siktiya	Maharajganj
4	Bhopatpur Bharathia	Lakri Nabiganj
5	Ramgarha	Daraunda

Season, physical target (in acre), number of beneficiaries and their socio-economic background are presented in Table 2.

**Table 2:** Season, physical target (in acre), number of beneficiaries and their socio-economic background

Seasons	Physical Target (in acre)	Number of beneficiaries	Socio-Economic background
<i>Rabi</i> 2020-21	623	1002	Majority of farmers are small & marginal with land holding held both in lease and own. All caste of the population of the village is included in the beneficiary list.
Summer 2021	250	521	Majority of farmers are small & marginal with land holding held both in lease and own. All caste of the population of the village is included in the beneficiary list.
<i>Kharif</i> 2021	595	996	Majority of farmers are small & marginal with land holding held both in lease and own. All caste of the population of the village is included in the beneficiary list.

Number of cropping season trailed were three which include *Rabi* 2020-21, Summer-2021 and *Kharif*-2021, technology adopted and their respective data recorded are presented in Table 3.

**RESULTS AND DISCUSSION**

During *Rabi* 2020-21, zero tilled wheat recorded higher grain yield in CRA demonstrated plot (40.35 q ha<sup>-1</sup>) whereas farmers’ practises (Control) recorded lower yield of 32.80 q ha<sup>-1</sup> with an increase of 23.02 per cent in yield was noticed in CRA plots over farmers’ practise (Keil *et al.* 2020) and 83.78 per cent increase in net income was noticed in CRA demonstrated plot (Table 3). Similarly Zero tilled lentil recorded higher grain yield and net returns than control (Farmers’ practise) which recorded 21.95 per cent increase in yield over farmers’ practise and 44.02 per cent increase in net income was



**Table 3:** Data recorded in CRA plots during *Rabi* 2020-21, Summer-2021 and *Kharif*-2021

Interventions	Grain yield (q/ha)		% increase over farmer practice	Net Return (INR)		% increase over farmer practice
	CRA Demonstration	Farmer practice		CRA Demonstration	Farmer practice	
<b>Rabi 2020-21</b>						
Wheat-Zero tillage	40.35	32.80	23.02	34000	18500	83.78
Lentil- Zero tillage	12.50	10.25	21.95	44250	30725	44.02
Wheat- Raised Bed Planting	42.25	32.80	28.81	32000	18500	72.97
Mustard-Raised bed planting	13.50	8.50	58.82	33500	24000	39.58
Maize-Raised Bed Planting	107.00	65.00	64.62	58500	36250	61.38
Maize with potato	102.50	86.00	19.19	53750	41225	30.38
Wheat-Community Irrigation	40.35	32.80	23.02	34000	18500	83.78
Wheat- Nutrient expert (LCC) based nutrient management	40.15	32.80	22.41	34000	18500	83.78
Potato- Potato based farming system	198.00	130.00	58.82	128000	75000	70.67
<b>Summer-2021</b>						
Moong bean- Zero Tillage	11.00	7.20	52.77	41650	30725	35.55
<b>Kharif-2021</b>						
Rice-Direct Seeded Rice (ZT + Drum seeder+Broadcasting)	41.00	32.00	25.00	25800	8800	193.18
Rice-Transplanting	40.00	32.00	25.00	22800	8800	159.09
Rice-Alternate wetting/ drying irrigation	40.00	32.00	25.00	22800	8800	159.09
Rice-Water harvesting & field bunding	38.00	32.00	18.75	20800	8800	136.36
Rice-Nutrient expert based nutrient management	40.00	32.00	25.00	22800	8800	159.09
Rice-Community Irrigation	41.00	32.00	28.13	25800	8800	193.18
Maize-Raised Bed Planting	44.00	38.00	15.79	35200	26400	33.33
Maize with Arhar Intercropping	44.00	38.00	15.79	35200	26400	33.33

noticed in CRA demonstrated plot (Zero tillage-Lentil) compared to control. Raised bed planting of wheat, mustard and maize recorded higher yield of 42.25, 13.50 and 107.00 q ha<sup>-1</sup>, respectively in CRA plot (Prabhavathi *et al.* 2015) than control (32.80, 8.50 and 65.00 q ha<sup>-1</sup>, respectively). Maize with potato intercropped CRA plot recorded 102.5 q ha<sup>-1</sup> yield while that of control recorded 86 q ha<sup>-1</sup> which showed 19.19 per cent increase in yield over farmers' practise. Community based irrigation and nutrient expert (LCC) based fertilizer application in wheat showed 23.02 and 22.41 per cent, respectively (Tek *et al.* 2021) over increment in yield and 83.78 per cent over increase in yield was observed in net returns in both treatments of CRA demonstrated plots than control.

Potato based farming system recorded grain yield of 198 q ha<sup>-1</sup> (CRA plot) and 130 q ha<sup>-1</sup> (Control) with satisfactory increase over yield (58.82 %) and net return (70.67 %). During Summer-2021, CRA

demonstrated moong bean plot recorded higher yield of grains (11.00 q ha<sup>-1</sup>) compared to control (7.22 q ha<sup>-1</sup>). Zero tillage practises reduces the consumption of energy and saves the time of farmer in tilling the entire land (Keil *et al.* 2020).

During *Kharif*-2021, DSR method of paddy sowing recorded higher yield of 41.00 q ha<sup>-1</sup> compared to farmers' practise (Jat *et al.* 2022) which recorded lower yield of 32.00 q ha<sup>-1</sup>. There was an increase of 25 per cent in yield over control of DSR method of rice cultivation. The DSR sown paddy crop reduced the labour and water requirement, preparation of transplanting bed, cost incurred towards transplanting of seedlings and time spent towards other activities of cultivation (Chauhan and Opena 2012). Similarly rice cultivated with alternate wetting and drying and transplanting methods recorded increase of yield by 25 per cent compared to farmers' practise (Ishfaq *et al.* 2020). Paddy field with water harvesting structure and field bundled



under CRA plots recorded higher yield of 38 q ha<sup>-1</sup> and increased net returns of ₹ 20800 per ha compared to control. Nutrient expert (LCC) based fertilizer application and Community irrigated plots also followed similar trend of increase in yield compared to control. Nutrient application through LCC reduces excessive application of fertilizer, which is more environment resilient and also curtails the money incurred over costly fertilizers. Community irrigation helps the marginal and small farmers to irrigate their crop during moisture stress situation. Raised bed planting and intercropping of maize fields with *arhar* showed increase in yield of 15.79 per cent over control and increment in net returns of 33.33 per cent. Raised beds acts as a drainage channel to drain excess water during floods and it also acts as a water storage structure which stores water *esp.* in root zone during dry spells. The results suggested that an adoption of CRA practises improved productivity of crops and also saved time and expenditure incurred towards cost of cultivation (Jat *et al.* 2022).

## CONCLUSION

Increasing scarcity of labour, water and energy and rising cost of production, along with climate variability are major challenges for the sustainability. The CRA practises save cost, energy, and also an input efficient and climate resilient technology compared to traditional planting methods.

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# Moisture Dependent Physical Properties of Psyllium Seeds for Different Varieties

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## ABSTRACT

The three varieties of psyllium seeds (VI-1, GI-3 and HI-5) were analysed for the effect of varying moisture content (6%, 12% and 18% w.b.) on the various physical properties. The physical properties are useful for the designing the cleaning, grading, conveying and size reduction equipment and storage structures. The dimensional properties like size and sphericity of psyllium seeds were ranged from 1.33 to 1.47 mm and 0.513 to 0.533. The gravimetric properties like, bulk density (550.07 to 585.54 kg/m<sup>3</sup>), true density (1206.78 to 1316.84 kg/m<sup>3</sup>) and porosity (54.06 to 56.75) were reduced as a function of moisture content except the thousand seed weight (1.52 to 1.82 g) which was found to be increased as the moisture increased. The frictional properties like static angle of repose (28.07 to 35.99°), coefficient of friction of glass (0.42 to 0.51), plywood (0.47 to 0.52) and galvanized iron (0.48 to 0.55) was also increased. The terminal velocity was also increased from 2.57 to 4.17 m/s as the moisture content was increased from 6% (w.b.) to 18% (w.b.). The individual effect of moisture content was found to be extremely significant ( $p < 0.001$ ) on all physical properties apart from non-significant effect on sphericity and porosity ( $p > 0.05$ ). Similarly, individual effect of variety was extremely significant on all physical properties ( $p < 0.001$ ). However, the interaction effect of moisture content and variety was only significant on the coefficient of static friction and terminal velocity.

## HIGHLIGHTS

- ① The article focuses on effect of both moisture content and variety on physical properties of psyllium seeds.
- ① The moisture content and variety both had significant effect on the physical properties of psyllium seeds.
- ① The size, thousand seed weight, angle of repose, coefficient of static friction and terminal velocity increased while bulk density, true density and porosity were decreased as the moisture content was increased.

**Keywords:** Psyllium, seeds, physical properties, moisture content, variety isabgol, isabgul

Psyllium is the one of the important medicinal plants grown in the western region of India. The psyllium plant is mainly sown during the December and harvested as a *rabi* crop in May or April. It is one of the most important export crops for India as it contributes to around 98 % of total world production. GI-1, GI-2, GI 3, VI-1, VI-3 RI-87, RI-89, AMB-2, MIB-4, HI-34, HI-2, HI-1, HI-5, JI-4 and Niharika are the major varieties of Isabgol grown in India (Anonymous, 2015). The psyllium

is mostly grown in the North Gujarat, Rajasthan, Haryana and some districts of Madhya Pradesh. In recent years, Rajasthan has been the leading producer of the psyllium seed while Gujarat is one of leading state in processing of psyllium seeds.

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During the post-harvest operation, psyllium seeds are cleaned and major impurities are winnowed by thresher and specific gravity separator. The cleaned seeds are mechanically dehusked by the emery stone dehusker at desired clearance to obtain the husk (Guo *et al.* 2009). The psyllium husk is the major source of dietary fibre which is used as a medicine to cure gut related diseases such viz., inflammation (Jalanka *et al.* 2019), diarrhea and irritable bowel syndrome (IBS) (Zhou *et al.* 2019), diabetes (Soltanian and Janghorbani 2019), helps in reduce obesity (Pal *et al.* 2019) and finds applications in different food industry such as a thickening agent in an ice-cream and functional food to enhance the textural properties in baked products (Gao *et al.* 2018). The mucilage extracted from psyllium husk can also be used as an alternate material for the coating of fruits and vegetables (Rehman *et al.* 2015) and for making a biodegradable packaging film (Ahmadi *et al.* 2012).

The knowledge of engineering properties of seeds is crucial for designing equipment and machinery for various post-harvest operations such as cleaning grading, conveying, milling. The size and shape are important in designing cleaning and separation machines, while bulk density, true density and porosity may require to be formulation of drying, aeration and storage structures. The terminal velocity of seeds is one of major consideration of designing in pneumatic conveyors. The angle of repose and coefficient of external friction is crucial for the bulk material handling equipment and storage structures.

There have been a few attempts to study the effect of moisture content on the engineering properties of psyllium seeds (Hashemifesharaki 2021) which significantly affected the engineering properties as a function of moisture content. While, physical properties of psyllium seeds were varied among the different varieties (Shah *et al.* 2020). However, no study has been found to show the effect of both moisture content and variety on the psyllium seeds. The present research aims to study the varietal influence as well as effect of moisture content on the physical, frictional and aerodynamic properties of psyllium seeds.

## MATERIALS AND METHODS

### Preparation of material

The psyllium seeds of three different variety i.e., VI-1, GI-3 and HI-5 were purchased from the regional medicinal research centre, Boriavi, Anand; Spices research station, Jagudan, Gujarat and Hissar agricultural University, Haryana, respectively. The seeds were manually cleaned through sieves and all the impurities i.e., dust, stones and chaff were removed to get desired cleaned seeds. The cleaned seeds were subject to different moisture content at 6%, 12% and 18% w.b. i.e., 6.38%, 13.63% and 21.95% in dry basis, respectively.

### Conditioning of seeds

The one kg of cleaned seeds was taken for each replication in the experiment. The one kg of each sample was placed in the metal trays for hot air oven drying (AOAC 2005) at  $105 \pm 5^\circ\text{C}$  to find the initial moisture content of seeds. The drying was carried out till the constant moisture content was achieved. The calculated amount of moisture was added to the dried seeds based on the equation given below. The care was taken to uniformly apply moisture in mist to the seeds. The seeds were packed in low density polyethylene bags and stored at  $8 \pm 2^\circ\text{C}$  till desired moisture was achieved. The seeds were conditioned at three moisture content i.e., 6.38%, 13.63% and 21.95% (d.b.).

$$Q = \frac{W_i \times (M_f - M_i)}{100 - M_f}$$

$Q$  is the calculated amount of water required to be added,  $W_i$  is the initial weight of psyllium,  $M_f$  is the initial moisture content (% d.b.) and  $M_i$  is the final moisture content (% d.b.) of psyllium seeds.

### Measurement of engineering properties of psyllium seeds

The seeds of three variety (VI-1, GI-3 and HI-5) and three moisture content (6%, 12% and 18% w.b.) were analysed for the different physical properties. The geometric properties like length, width, thickness, size, sphericity was replicated ten times. The dimensions of length (L), width (W) and Thickness (T) in millimetre were measured using a digital vernier calliper having an accuracy of 0.01 mm.



The size and sphericity were calculated using the following formula (Mohsenin 1986):

$$\text{Size (mm)} = (L \times W \times T)^{1/3}$$

$$\text{Sphericity} = \frac{(L \times W \times T)^{1/3}}{L}$$

The gravimetric properties like thousand seed weight, bulk density, true density, porosity was replicated thrice. Thousand seed weight taken by manually counting the thousand seed and weighing the seeds in an electronic balance with an accuracy of 0.001 g. The bulk density was measured by taking the mass of sample filled in a rectangular box of dimension 10.533 cm × 10.163 cm × 9.792 cm from a constant height. The bulk density (kg/m<sup>3</sup>) was calculated using the following formula (Mohsenin 1986).

Bulk density (kg/m<sup>3</sup>) =

$$\frac{\text{Weight of psyllium seeds (g)}}{\text{Total volume occupied by psyllium seeds (cm}^3\text{)}} \times 1000$$

The true density was measured using the liquid displacement method using the toluene because of its little or non-absorptivity of seed with the toluene. Specific gravity of toluene was taken as 0.866 g/cc. The pycnometer was used to measure the true volume of displaced toluene for the known weight of added sample. The true density was calculated and expressed in kg/m<sup>3</sup> using the following formula (Mohsenin 1986):

True density (kg/m<sup>3</sup>) =

$$\frac{\text{Weight of psyllium seeds (g)}}{\text{True volume occupied by psyllium seeds (cm}^3\text{)}} \times 1000$$

The porosity was calculated using the from the values of true and bulk density using the relationship given below:

$$\text{Porosity (\%)} = \left( 1 - \frac{\text{Bulk density}}{\text{True density}} \right) \times 100$$

The frictional properties viz. emptying static angle of repose and coefficient of static friction on glass, plywood and galvanized iron sheets was also measured with three replications. The static angle

of repose was measured by filling the box having the circular platform with the psyllium seeds from a constant height to form a stable cone. The extra seeds were removed out by the discharge hole. The height of the stable cone of seeds developed on the circular platform by the measuring scale. The static angle of repose was measured with the following formula:

$$\theta = \tan^{-1} \left( \frac{2H}{D} \right)$$

The coefficient of static friction was measured by the inclined plane method (Mohsenin 1986) for three different surfaces i.e., glass, galvanized iron sheet, plywood. The seeds were filled up to the top of the bottomless container placed at the end of the plain and plain was tilted slowly till the container starts sliding over the plain. The horizontal and vertical distances were measured and coefficient of static friction was measured using the following formula:

Coefficient of static friction ( $\mu$ ) =

$$\tan \theta = \frac{\text{Vertical distance}}{\text{horizontal distance}}$$

The terminal velocity was measured by suspending the psyllium seeds in the air column and measuring the velocity of air to float the psyllium seed by the digital anemometer (Model: AVM-03; Mfg. by Work Zone) with capability of measuring velocity of 0-45 m/s ± 3%.

## STATISTICAL ANALYSIS

The various experimental responses for the nine treatment combinations of three moisture content and three variety (VI-1, GI-3, HI-5) were statistically analysed using the Factorial Completely Randomized Design (FCRD). The data of experiments were feed into Design Expert software (v13; State-Ease USA) and the ANOVA table with the significance value was generated.

## RESULTS AND DISCUSSION

### Seed Moisture content

The initial moisture content of cleaned seed before drying was found to be 8.47% (w.b.). The average moisture content of treated sample for VI-1 variety



was  $6.25 \pm 0.30\%$ ,  $11.86 \pm 0.26\%$  and  $17.76 \pm 0.33\%$  (w.b.). For GI-3 variety, moisture content of psyllium seeds was found to be  $6.36 \pm 0.13\%$ ,  $12.09 \pm 0.46\%$  and  $17.82 \pm 0.44\%$  (w.b.). Similarly, moisture content of HI-5 variety of seeds was found to be  $6.13 \pm 0.40\%$ ,  $11.80 \pm 0.46\%$  and  $17.88 \pm 0.33\%$  (w.b.). These shows that the moisture content of treated psyllium seeds was in the acceptable range of variation of 0.5% for all the treatment combinations. The measurement of different engineering properties was carried out at these levels to determine effect of moisture content on psyllium seeds.

### Size

The size of psyllium seeds was found to be varying among different variety and the moisture content (Figure 1(a)). Initially, size of the VI-1 variety was found to be the smallest at 1.33 mm while, the variety HI-5 had the largest seed size of 1.39 mm among three varieties. The size of VI-1 variety was found to be increased 2.25 % (1.36 mm) at 12% and 5.26% (1.40 mm) at 18% (w.b.) moisture content, respectively. The GI-3 variety of seeds were almost similar in the size at 1.34 mm and increased 3.73 % larger (1.39 mm) at 12% and 7.46% (1.44 mm) at 18% (w.b.). However, HI-5 were the largest in the size among all three varieties at 1.39 mm and found to be increasing in the size with an 3.59% (1.44 mm) increase at 12% moisture content and 5.75% (1.49 mm) at 18% (w.b.) moisture content. Similar increase in size as the function of moisture content (1.29 to 1.36 mm) was observed by Hashemifesharaki (2021). Increase in the size was observed because of absorbance of moisture led to swelling of psyllium seeds and increase in the axial dimensions (Hashemifesharaki 2021). The individual effect of moisture content and the variety were both found to be extremely significant ( $p < 0.001$ ) (Table 1). However, interaction effect of moisture content and variety was found to be non-significant ( $p > 0.05$ ).

### Sphericity

The sphericity of psyllium seeds was found to be in the range of 0.513 to 0.533 (Fig. 1(b)). The sphericity of VI-1 varied from 0.519 (12% w.b.) to 0.527 (6% w.b.). The sphericity of GI-3 seeds was found to be decreased from 0.521 (6% w.b.) to 0.513 (18% w.b.). While, seeds of HI-5 variety showed slight increase

in sphericity from 0.527 (6% w.b.) to 0.533 (18% w.b.). The sphericity of HI-5 variety was to be highest 0.533 at 18% (w.b.) while the GI-3 had the smallest sphericity 0.513 (18% w.b.) Sphericity of psyllium seeds was in the range of sphericity observed by Ahmadi *et al.* (2021). Ahmadi *et al.* (2021) reported that sphericity of psyllium seeds among different varieties did not vary. While, Hashemifesharaki (2021) reported that increasing moisture content from 4.32% (d.b.) to 20.36% (d.b.) led to increase of sphericity from 51.70% to 52.50%. Sphericity of the psyllium seeds among did not vary significantly among different moisture content ( $p > 0.05$ ) (Table 1). The varietal effect of psyllium seed on sphericity was also found to be non-significant ( $p > 0.05$ ).

### Thousand seed weight

The thousand seed weight is essential in designing the milling equipment. Initially, psyllium seeds of VI-1 and GI-3 were similar in thousand seed weight at 1.52 and 1.53 g, respectively; while the thousand seed weight of HI-5 was considerably higher (1.68 g) at 6% (w.b.) at 6% (w.b.). The increase in a moisture led to significant increase in the thousand seed weight among all three varieties. For the VI-1 variety, there was an increase of 3.17% (1.57 g) and 7.27% (1.63 g) at 12% and 18% (w.b.) moisture content, respectively. The thousand seed weight of GI-3 variety was increased to 7.18% (1.64 g) and 9.80% (1.68 g). Similar observation was found in the HI-5 variety where, the weight was increased to 3.57% (1.74 g) and 8.33% (1.82 g) increased at 12% and 18% (w.b.) moisture content, respectively. Hashemifesharaki (2021) observed similar increase in thousand seed weight 1.24 to 1.50 g. This could have happened due to absorption of moisture into the husk of psyllium seeds which led to increase in the weight. The individual effect of moisture content and variety were both found to be extremely significant on the thousand seed weight (Table 1). The interaction effect of moisture content and variety had no significant on thousand seed weight.

### Bulk density

The bulk density of psyllium seeds was found to be decreasing as the moisture content was increased among all three varieties (Figure 1(d)). At 6% (w.b.) moisture content, the GI-3 variety had the highest

bulk density of 585.54 kg/m<sup>3</sup> while, the VI-1 had the lowest bulk density of 569.60 kg/m<sup>3</sup> among all varieties. While the bulk density of HI-5 variety was found to be 576.32 kg/m<sup>3</sup>. In VI-1 variety, there was a decrease of 1.55% (560.76 kg/m<sup>3</sup>) at 12% w.b. and 3.54% (550.07 kg/m<sup>3</sup>) at 18% w.b. from initial moisture content. The GI-3 variety showed a 3.10% (567.33 kg/m<sup>3</sup>) decrease at 12% (w.b.) moisture content and 5.17% (555.26 kg/m<sup>3</sup>) decrease at 18% (w.b.) moisture content. The decrease in bulk density of HI-5 was found to be 1.73% (566.31 kg/m<sup>3</sup>) and 3.94% (553.60 kg/m<sup>3</sup>) at 12% and 18% (w.b.) moisture content, respectively. Decrease in bulk density of pigeon pea grain as a function of moisture content was also observed by Sangani and Davara (2013). Hashemifesharaki (2021) also reported decrease of bulk density in the psyllium seeds. This decrease could be attributed to the increase in mass was lower than the volumetric expansion of seeds due to absorption of moisture. The individual effect of moisture content on bulk density was found to be extremely significant ( $p < 0.001$ ). The individual effect of variety on bulk density was also found significant ( $p < 0.05$ ) (Table 1). However, the combined effect of moisture and variety was found non-significant on the bulk density of psyllium seeds ( $p > 0.05$ ).

### True density

The experimental results among all three varieties indicates that the true density of psyllium seeds was also decreased as the moisture content was increased (Fig. 1(e)). At 6% (w.b.) moisture content, the VI-1 variety had the highest true density of 1316.84 kg/m<sup>3</sup> while, the HI-5 had the lowest bulk density of 1288.33 kg/m<sup>3</sup> among all varieties. The true density of GI-3 variety was found to be 1302.80 kg/m<sup>3</sup>. In VI-1 variety, there was a decrease of 2.25% (1287.22 kg/m<sup>3</sup>) at 12% w.b. and 4.27% (1260.58 kg/m<sup>3</sup>) at 18% w.b. from initial moisture content. The GI-3 variety showed a 3.84% (1252.77 kg/m<sup>3</sup>) decrease at 12% (w.b.) moisture content and 7.22% (1208.78 kg/m<sup>3</sup>) decrease at 18% (w.b.) moisture content. The decrease in true density of HI-5 was found to be 2.65% (1254.19 kg/m<sup>3</sup>) and 6.33% (1206.78 kg/m<sup>3</sup>) at 12% and 18% (w.b.) moisture content, respectively. The decrease in pore space and volumetric expansion of psyllium seeds led to decrease in the true density of psyllium seeds (Hashemifesharaki 2021). The individual effect of

both moisture content and variety had an extremely significant effect on the true density ( $p < 0.001$ ) (Table 1). However, the combined effect of moisture and variety was found non-significant on the true density of psyllium seeds ( $p > 0.05$ ).

### Porosity

The effect of moisture content on the porosity was found to be negligible (Figure 1(f)). There was a very slight decrease in the porosity in all three varieties as the moisture content progressed. The highest porosity of psyllium seeds at 6% w.b. moisture was found in the VI-1 variety (56.75%). The GI-3 had the least porosity of 55.05% while the porosity of the HI-5 variety of seeds was similar to the GI-3 (55.26%). There was 0.55% and 0.73% decrease in VI-1 variety at 12% and 18% moisture content. The GI-3 variety faced a decrease of 0.62% and 1.80%. The highest decrease in the porosity was observed in the HI-5 variety with a decrease of 0.78% and 2.08% at 12% and 18% (w.b.) moisture content. The intercellular voids of seeds filled with the moisture and increased volumetric expansion could be the reason for decrease in porosity (Hashemifesharaki 2021). The individual effect of moisture content was found to be non-significant ( $p > 0.05$ ) on the porosity while the variety had the extremely significant effect on the porosity ( $p < 0.001$ ). The interaction effect of moisture content and variety was also found to be non-significant (Table 1).

### Static angle of repose

Static angle of repose was found to be increasing irrespective of any variety as the moisture content was increased (Fig. 1(g)). The VI-1 variety had the lowest static angle of repose of 28.04° while the HI-5 had the highest angle of 30.96°. The static angle of repose of GI-3 was observed to be 28.32°. Increasing the moisture content from 6% to 12% and 18% moisture content led to an increase of 12.72% and 20.57%, respectively in the VI-1 variety. The intermediate increase of 12.14% (31.76°) and 22.54% (34.76°) was found at 12% and 18% (w.b.) moisture content in the GI-3 variety. The HI-5 variety showed least increase of 5.39% (32.63°) at 12% (w.b.) and 16.24% (35.99°) at 18% (w.b.) among all varieties. This increase in the static angle of repose could be due to increased plasticity of seed surfaces and stickiness between the surfaces at seeds



of higher moisture content (Garavand *et al.* 2009). The individual effect of both moisture content and variety had an extremely significant effect on the static angle of repose ( $p < 0.001$ ) (Table 1). However, the combined effect of moisture and variety was found non-significant on the static angle of repose of psyllium seeds ( $p > 0.05$ ).

### Coefficient of static friction

The coefficient of static friction was observed on three different surfaces glass, plywood and galvanized iron sheet. The observation showed that the coefficient of static friction of glass was found to be least, coefficient of static friction of plywood was intermediate and the highest coefficient of static friction were observed in the galvanized iron sheet. The individual analysis of all three surfaces for different moisture content and variety was also observed.

At initial 6% moisture content, the coefficient of static friction of glass was found to be lowest in the HI-5 variety (0.420) (Fig. 1(h)). The coefficient of static friction of glass in VI-1 and GI-3 were found to be similar at 0.460 and 0.453, respectively. The coefficient of static friction of glass was found to be increasing as the moisture content was increased to 12% and 18% (w.b.); the VI-1 variety showed a least increase in the coefficient of static friction at 1.03% and 6.27%. The GI-3 variety showed the highest increase of 3.41% at 12% moisture content and 13.34% at 18% moisture content. The increase in coefficient of static friction in glass for HI-5 variety was observed to be 4.43% and 6.66% at 12% (w.b.) and 18% moisture content, respectively.

The coefficient of static friction of plywood did not vary too much among all three varieties (Figure 1(i)). The lowest coefficient of static friction of plywood (0.469) was found in the VI-1 variety, while the highest coefficient of static friction of plywood was found in the HI-5 variety at initial 6% moisture content. The coefficient of static friction of plywood was found to be increased irrespective of all varieties. The increase in VI-1 variety were in the range of 1.79% (0.477) and 9.61% (0.514) at 12% and 18% (w.b.) moisture content. The GI-3 had the highest increase of 2.55% (0.485) at 12% (w.b.) and 10.08% (0.521) after 18% (w.b.) moisture content. The lowest reduction in the coefficient of static friction

of plywood was observed in the HI-5 variety with the 4.31% (0.500) at 12% w.b. and 6.12% (0.509) at 18% (w.b.) moisture content.

The coefficient of static friction of galvanized iron was found to be highest among all three surfaces (Fig. 1(j)). Initially at 6% moisture content, the coefficient of static friction of galvanized iron of VI-1, GI-3 and HI-5 variety was 0.484, 0.488 and 0.511, respectively. The highest increase in the coefficient of static friction of galvanized iron was observed in the GI-3 variety with an increase of 4.52% (0.488) and 10.93% (0.541) at 12% and 18% moisture content, respectively. The HI-5 variety of seeds were found to least increased in the coefficient of static friction of galvanized iron with an increase of 2.92% (0.526) and 7.37% (0.549). The increase in the coefficient of static friction of galvanized iron for VI-1 variety was in the range of 3.27% (0.500) and 10.72% (0.536).

The increase coefficient of static friction might have happened due to the increased adhesion of mucilaginous surface of psyllium husk between the seeds at higher moisture and different material surfaces (Hashemifesharaki 2021). Statistical analysis of coefficient of static friction of glass, plywood, galvanized iron showed that the individual effect of moisture content and variety had extremely significant effect ( $p < 0.001$ ) irrespective of contact surfaces (Table 1). The interaction effect of moisture content and variety on coefficient of static friction for glass and plywood was also found to be significant ( $p < 0.05$ ). While in the case of galvanized iron, the interaction effect of moisture content and variety was found to be non-significant ( $p > 0.05$ ).

### Terminal velocity

The terminal velocity of psyllium seeds among different varieties were in the range of 2.57 to 4.17 m/s (Fig. 1(k)). The lowest terminal velocity was observed in the VI-1 variety at 2.57 m/s, while the HI-5 variety had the highest terminal velocity of 2.83 m/s at 6% (w.b.) moisture content. The terminal velocity of GI-3 variety was recorded as 2.77 m/s. There was a significant increase in the terminal velocity as the moisture content was increased from 6% to 12% and 18%, respectively. The increase in terminal velocity of VI-1 variety was increased 23.78% (3.19 m/s) and 46.68% (3.78 m/s) post increase in moisture content to 12% and



Table 1: Effect of moisture content and different variety on the physical properties of psyllium seeds

Effect	Size (mm)	Sphericity	Bulk density (kg/m <sup>3</sup> )	True density (kg/m <sup>3</sup> )	Porosity (%)	Thousand seed weight (g)	Emptying angle of repose (degree)	Coefficient of static friction on Glass	Coefficient of static friction on Plywood	Coefficient of static friction on GI	Terminal velocity (m/s)
<b>Moisture content (M)</b>											
6%	1.354	0.526	577.155	1302.659	55.687	1.578	29.118	0.445	0.474	0.494	2.742
12%	1.399	0.523	564.802	1264.727	55.327	1.649	32.012	0.458	0.488	0.512	3.279
18%	1.441	0.524	552.977	1225.382	54.836	1.708	34.863	0.484	0.515	0.542	3.925
S. Em±	0.011	0.003	2.163	5.909	0.315	0.007	0.220	0.003	0.002	0.002	0.031
C. D. at 5%	0.03***	NS	6.427***	17.693***	NS	0.021***	0.653***	0.008***	0.007***	0.007***	0.092***
<b>Variety (V)</b>											
VI-1	1.366	0.523	560.145	1288.216	56.505	1.574	31.185	0.471	0.486	0.507	3.198
GI-3	1.392	0.518	569.378	1254.784	54.608	1.615	31.614	0.478	0.493	0.513	3.314
HI-5	1.436	0.532	565.411	1249.768	54.737	1.746	33.195	0.436	0.496	0.529	3.434
S. Em±	0.011	0.003	2.163	5.909	0.315	0.007	0.220	0.003	0.002	0.002	0.031
C. D. at 5%	0.03***	NS	6.427*	17.693***	0.937***	0.021***	0.653***	0.008***	0.007*	0.007***	0.092***
<b>Interaction (M*V)</b>											
S. Em±	0.018	0.006	3.747	10.235	0.546	0.013	0.381	0.005	0.004	0.004	0.053
C. D. at 5%	NS	NS	NS	NS	NS	NS	NS	0.0142*	0.013*	NS	0.1588*
C.V. %	4.16	3.44	1.149	1.402	1.710	1.312	2.061	1.788	1.480	1.370	2.792

Number of replications, n = 3

\*\*\* Significant at p &lt; 0.001

\*\* Significant at p &lt; 0.01

\* Significant at p &lt; 0.05

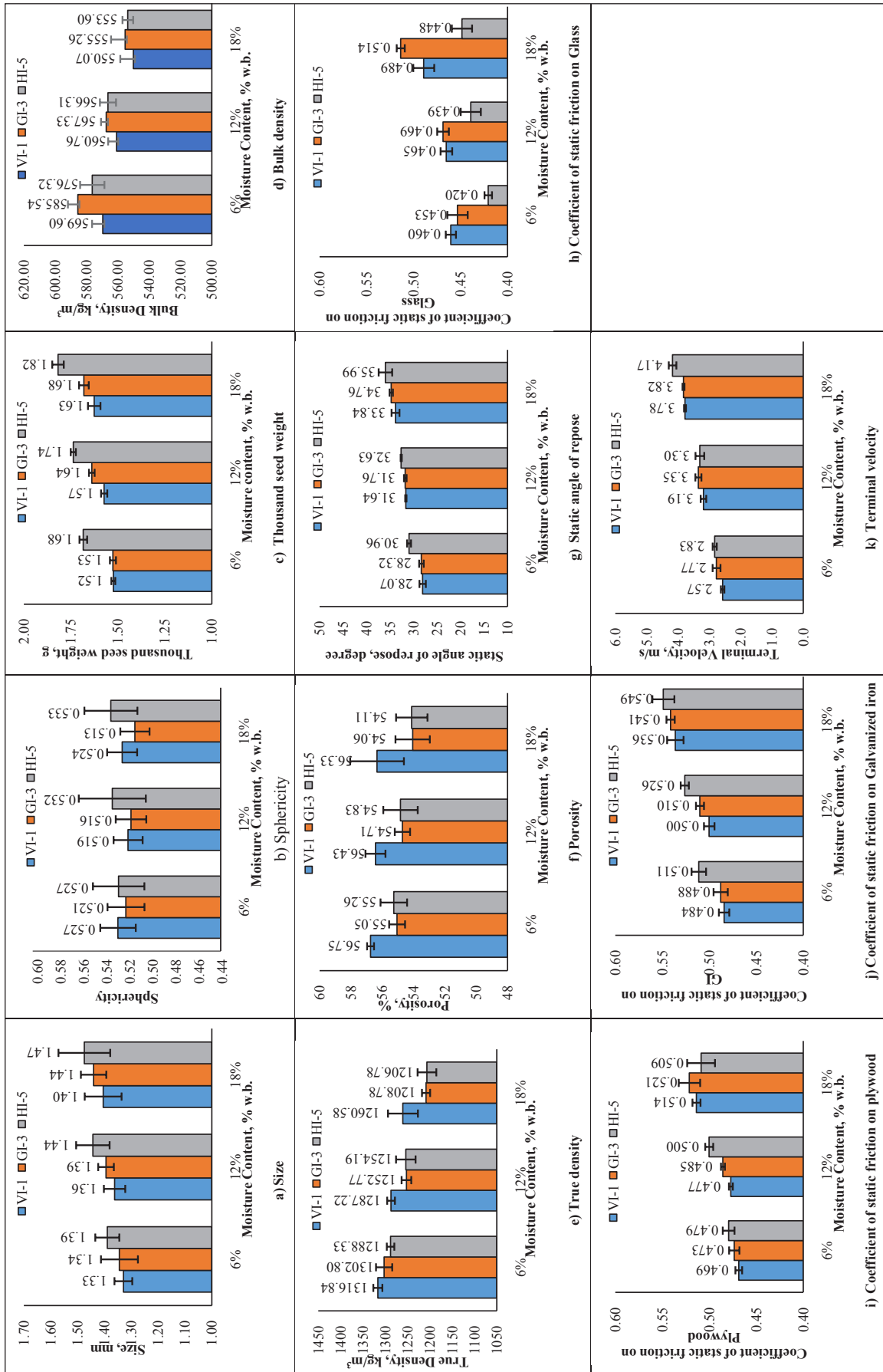


Fig. 1: Effect of moisture content and different varieties on various engineering properties of psyllium seeds



18% (w.b.), respectively. The GI-3 variety showed slightly lower increase at 20.95% (3.35 m/s) at 12% moisture content and 38.12% (3.82 m/s) increase at 18% (w.b.) moisture content. The terminal velocity of HI-5 variety was found to increasing at 16.78% (3.30 m/s) and 47.62% (4.17 m/s) at 12% and 18% (w.b.) moisture content, respectively. This increase in the terminal velocity could be attributed to increase in the mass of seeds due to increase in moisture content. Similar observation in safflower seeds (Khanahmadzadeh *et al.* 2021), sesame seeds (Garavand *et al.* 2009) and psyllium seeds (Hashemifesharaki 2021) were observed. The individual effect of both moisture content and variety on terminal velocity was found to be extremely significant ( $p < 0.001$ ). The combined effect of moisture content and variety was also found significant ( $p < 0.05$ ) on terminal velocity of psyllium seeds (Table 1).

## CONCLUSION

The analysis of moisture content on the different varieties resulted in the variation of physical properties of psyllium seeds. The dimensional properties like size, sphericity, thousand seed weight, angle of repose, coefficient of static friction and terminal velocity were found to be increased as the moisture content was increased. The gravimetric properties of bulk density, true density and porosity were observed to be decreased as the function of moisture content. It can be concluded that moisture content and variety both had the significant effect on the psyllium seeds. These observations would be helpful to develop models for predicting the physical response of seeds at varying moisture content and in the design of various post-harvest equipment of psyllium seeds.

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# Management of *Plutella xylostella* on Cauliflower Crop Through Novel Group of Insecticides

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## ABSTRACT

The use of insecticides on cruciferous vegetables is one of the major control measures used by farmers. Due to the short life cycle of Diamondback moth *P. xylostella*, it has developed resistance to many insecticides. To test the efficacy of insecticides on Diamondback moth (DBM) on cauliflower crop, ten insecticides were evaluated against DBM on cauliflower in *rabi*, 2017-18 and 2018-19. The cumulative (2017-18 and 2018-19) mean per cent *P. xylostella* larval control was highest in plots treated with tolfenpyrad (91.61%) followed by spinetoram (91.41%). The bifenthrin (71.49%) and acephate (69.57%) treated plots showed least per cent efficacy. The results revealed that the lesser sensitivity and efficacy of older insecticides to DBM. Regular monitoring, efficacy trials, renewal and recommendation of new molecules is the need of the hour as a part of chemical management.

## HIGHLIGHTS

- The most effective insecticide against the DBM was tolfenpyrad followed by the spinetoram in the two season.
- The bifenthrin followed by acephate shown the least per cent efficacy against the DBM.
- The DBM was most susceptible to the novel group of insecticides compared to the other group

**Keywords:** Diamondback moth, cauliflower, insecticides and efficacy

The Diamondback moth *P. xylostella* is the most destructive and distributed pest of cruciferous crops in the world (Meyrick 1928 and Talekar and Shelton 1993). Larval feeding results in skeletisation of leaves leaving behind the veins and the damage at seedling stage affect the head formation. The damage to cauliflower head also results in discoloration of the curd and rejection of the curds in the market (Capinera 2002; Shelton 2004).

In many countries, the *P. xylostella* has developed many fold of resistance to many synthetic insecticides in the field and to the bacterial insecticide *Bacillus thuringiensis* (Talekar *et al.* 1990; Tabashnik *et al.*

1987 and Meghana *et al.* 2017). The resistance is also reported to some market trending products like chlorantraniliprole, spinosad, beta-cypermethrin, chlorfenapyr and diafenthiuron (Jiang *et al.* 2015) due to characters like short generation time, high reproductive capacity and heredity (Shanmugapriya *et al.* 2018).

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The development of resistance through selection pressure is never an ending process. The introduction and testing of novel insecticides is necessary to know the comparative efficacy, cost benefit ratio and for region specific recommendation.

## MATERIALS AND METHODS

Field trials were conducted in farmer's field near Narasingapuram (Chandragiri mandal) village for testing the effective insecticide for management of population of *P. xylostella* in *rabi* season of 2017-18 and 2018-19. Novel insecticides to manage resistance were selected based on the survey conducted earlier from the farmer fields.

### Details of the Experiment

#### Crop and variety

Cauliflower variety Dhaval (East-West Seed India Pvt. Ltd.) (popular variety among farmers) was selected to transplant in field to study the efficacy of novel insecticides in controlling the *P. xylostella*. Seedlings of thirty days old were procured from Nursery located near Ramasamudram and transplanted in the field.

#### Preparation of the main field

The land measured was ploughed thoroughly with tractor drawn cultivator. Weeds and stubbles were removed and land was levelled using tractor drawn levelling plank.

#### Layout of field trial

The field was laid out in a randomised block design (RBD) with ten treatments including the untreated control and replicated thrice. The gross plot size of 38 m × 25 m was selected. The plot size of 8.00 m × 3.60 m was prepared with bunds all around the plots. Replications were separated with a gap of 0.5 metre for irrigation channels (Fig. 1).

#### Transplantation

The seedlings of thirty days old received from the nursery were transplanted immediately after irrigation during *rabi* season of 2017-18 and 2018-19. The seedlings were transplanted in line with a spacing of 60×45 cm (60 cm between the rows and 45 cm within the row). Gap filling was done after one week to obtain uniform population (Fig. 1).

### Weeding and Irrigation

Weeding was carried out using manual labour at ten days interval after transplantation. Irrigation was provided in two days interval and after weeding and earthing up was carried out.

### Fertilizer application

A recommended dose of fertilizer dose of 32:40:32 kg of NPK/ha was applied to trial field in the form of urea, single super phosphate and muriate of potash. The required quantities of each of these fertilizers to the laid out plot were calculated and then applied uniformly. Nitrogen was applied in three split doses at 30, 60 and 80 days after transplanting. The phosphorus and muriate of potash applied as basal dose. To avoid the deficiency of boron, spraying of borax was done two times at two weeks after treatment and two weeks before flowering at the rate of 3 g L<sup>-1</sup>. Inter-cultivation was done at 20 and 25 days after transplanting and further carried out whenever necessary.

### Treatments

Nine insecticides such as bifenthrin, emamectin benzoate, tolfenpyrad, cyantranilprole, spinosad, chlorantranilprole, spinetoram, novaluron and acephate (recommendation of the package of practice of the Dr. Y. S. R. Horticultural University) along with untreated control were imposed to test the efficacy of each insecticide. The treatments were selected based on survey conducted and novelty of the insecticides (Table 1 and Fig. 1).

### Preparation and application of insecticides

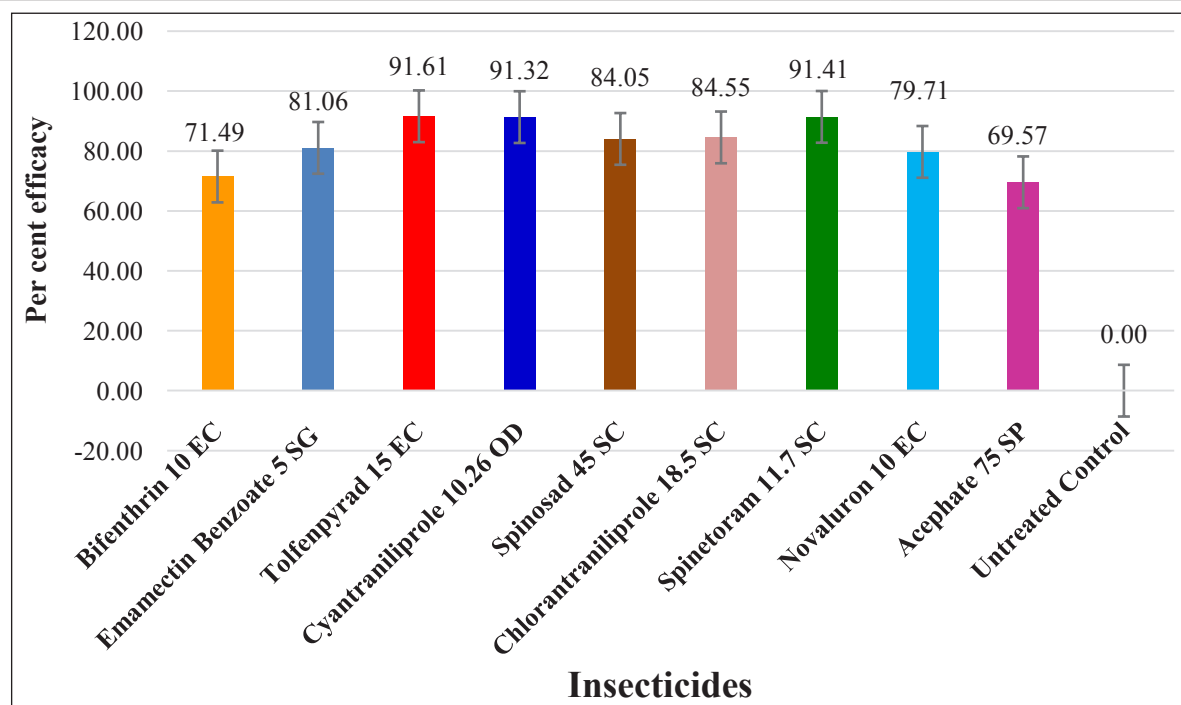
Insecticides were sprayed when the level of incidence reached economic threshold level (ETL). Required quantities of selected insecticides were measured with the help of measuring cylinder and mixed with required quantities of water to get desired dilution and sprayed with a high volume knapsack sprayer. After every application of each of the treatments, the sprayer was thoroughly washed and rinsed twice with water and used for further applications (Fig. 1).

### Data observations

In the field five randomly selected plants (leaving border plants) were labelled using yellow tag and

**Table 1:** Details of insecticides imposed to test the efficacy against DBM, *P. xylostella* during *rabi*, 2017-18 and 2018-19

Sl. No.	Treatment	Dosage	Trade Name	Source of supply
T1	Bifenthrin 10 EC	1.5 mL/L	Aalstar	Agastya Agro Limited
T2	Emamectin Benzoate 5 SG	0.4 g/L	Proclaim	Syngenta India Limited
T3	Tolfenpyrad 15 EC	2 mL/L	Keefun	PI Industries Limited
T4	Cyantraniliprole 10.26 OD	0.3 mL/L	Benevia	DuPont India Private Limited
T5	Spinosad 45 SC	0.3 mL/L	Taffin	Rallis India Limited
T6	Chlorantraniliprole 18.5 SC	0.3 mL/L	Coragen	DuPont India Private Limited
T7	Spinetoram 11.7 SC	1 mL/L	Summit	Rallis India Limited
T8	Novaluron 10 EC	1 mL/L	Rimon	Indofil Industries Limited
T9	Acephate 75 SP	1 g/L	Asataf	Rallis India Limited
T10	Untreated control	—	—	—

**Fig. 1:** Cumulative mean efficacy of certain insecticides against larvae of *P. xylostella* in cauliflower during *rabi*, 2017-18 and 2018-19

the data on incidence of *P. xylostella* larvae and number of leaf damaged per plant recorded before and after treatment of the insecticide. The data was recorded at 3, 5 and 7 days after insecticide application (DAA). Per cent efficacy was calculated using formulae.

Percent efficacy =

$$\frac{\text{Larval count in treated plot} - \text{Larval count in control plot}}{\text{Larval count in control plot}} \times 100$$

## Yield

The crop was harvested when all the curds got matured. Plot wise curd yield was recorded by harvesting crops leaving two border rows from all sides. The yield obtained from various treatments were expressed as ton ha<sup>-1</sup> (Fig. 1).

## STATISTICAL ANALYSIS

The data recorded on pest population and extent of insect pest damage obtained from field experiments were subjected to arcsine transformation. The treatment variations were tested for significance



by 'F test' using software SPSS (version 21). The standard error of means SE (m)  $\pm$  and Critical Differences (CD) at 5 per cent level of significance were calculated following the standard procedure and treatment means were compared using CD. Based on the statistically analyzed data, the results of the investigation were interpreted and conclusion was drawn.

## RESULTS AND DISCUSSION

### Efficacy of insecticides against larvae of *P. xylostella* during *rabi*, 2017-18

#### At 3 days after application of insecticides

The larval count in pre-treatment were in the range of 5.00 to 6.73 per plant. At 3 Days After Application (DAA), the number of larvae was controlled more effectively in treatments tolfenpyrad, spinetoram, cyantraniliprole and chlorantraniliprole *viz.*, 91.31, 91.06, 89.88 and 84.45 per cent, respectively and on par with each other followed by spinosad, emamectin benzoate, novaluron and bifenthrin with on par per cent control of 81.35, 81.06, 78.61 and 78.41 per cent respectively and significantly less control was observed in treatment acephate with 72.90 per cent (Table 2).

#### At 5 days after application of insecticides

At 5 DAA, the treatments tolfenpyrad, spinetoram and cyantraniliprole shown significantly higher efficacy followed by chlorantraniliprole, novaluron, spinosad and emamectin benzoate with 91.15, 91.60, 90.54 and 83.81, 82.75, 81.54 per cent reduction of larvae, respectively. The least reduction of larvae was observed in acephate and bifenthrin 73.45 and 72.39 per cent, respectively (Table 2).

#### At 7 days after application of insecticides

At 7 DAA, spinetoram (92.03%), cyantraniliprole (90.88%) and tolfenpyrad (90.73%) were found to be the most effective treatments against DBM larvae and followed by spinosad (86.42%) and chlorantraniliprole (85.77%). These treatments followed by emamectin benzoate (82.27%) and novaluron (82.21%). The bifenthrin (73.42%) and acephate (72.62%) were found to be least effective against *P. xylostella* (Table 2).

The mean values of per cent efficacy of different insecticides over untreated control against larvae of *P. xylostella* at 3, 5 and 7 days were calculated. The mean per cent efficacy was highest in spinetoram (91.21%), tolfenpyrad (91.06 %) and cyantraniliprole (90.79%) without any significant difference. The next best insecticides were chlorantraniliprole (85.28%)

**Table 2:** Efficacy of certain insecticides against *P. xylostella* larval population in cauliflower during *rabi*, 2017-18

Sl. No.	Treatment	Dosage	PTC (No. of larvae per plot)	Per cent efficacy over control			
				3 DAA	5 DAA	7 DAA	Mean
T1	Bifenthrin 10 EC	1.5 mL/L	5.13	78.41 (62.32) <sup>cd</sup>	72.39 (58.32) <sup>c</sup>	73.42 (58.96) <sup>d</sup>	74.74 (59.86) <sup>d</sup>
T2	Emamectin Benzoate 5 SG	0.4 g/L	5.40	81.06 (64.23) <sup>cd</sup>	81.54 (64.72) <sup>b</sup>	82.27 (65.12) <sup>c</sup>	81.62 (64.62) <sup>c</sup>
T3	Tolfenpyrad 15 EC	2 mL/L	6.73	91.31 (72.89) <sup>a</sup>	91.15 (72.70) <sup>a</sup>	90.73 (72.27) <sup>a</sup>	91.06 (72.60) <sup>a</sup>
T4	Cyantraniliprole 10.26 OD	0.3 mL/L	5.33	89.88 (71.47) <sup>ab</sup>	91.60 (73.25) <sup>a</sup>	90.88 (72.47) <sup>a</sup>	90.79 (72.34) <sup>a</sup>
T5	Spinosad 45 SC	0.3 mL/L	6.07	81.35 (64.88) <sup>c</sup>	82.75 (65.56) <sup>b</sup>	86.42 (68.43) <sup>b</sup>	83.51 (66.08) <sup>bc</sup>
T6	Chlorantraniliprole 18.5 SC	0.3 mL/L	5.53	84.45 (66.86) <sup>ab</sup>	85.63 (67.74) <sup>b</sup>	85.77 (67.92) <sup>bc</sup>	85.28 (67.45) <sup>b</sup>
T7	Spinetoram 11.7 SC	1 mL/L	5.20	91.06 (72.76) <sup>a</sup>	90.54 (72.13) <sup>a</sup>	92.03 (73.65) <sup>a</sup>	91.21 (72.77) <sup>a</sup>
T8	Novaluron 10 EC	1 mL/L	5.00	78.61 (62.67) <sup>cd</sup>	83.81 (66.36) <sup>b</sup>	82.21 (65.09) <sup>c</sup>	81.54 (64.59) <sup>c</sup>
T9	Acephate 75 SP	1 g/L	4.93	72.90 (58.63) <sup>d</sup>	73.45 (59.00) <sup>c</sup>	72.62 (58.45) <sup>d</sup>	72.99 (58.69) <sup>d</sup>
T10	Untreated control		5.40	—	—	—	—
F Test of Significance @ df=9				Sig.***	Sig.***	Sig.***	Sig.***
CD (P = 0.05)				5.52	3.32	2.62	2.11
SE(M)				1.86	1.12	0.88	0.71

PTC – Pre Treatment Count, DAA – Days After Application; Values are the means of three replication; Values in the parentheses are arcsine transformed values; Means followed by different letters are significantly different by DMRT ( $P \leq 0.05$ , LSD); \*\*\*= $P \leq 0.005$ .



and spinosad (83.51%) followed by emamectin benzoate (82.27%) and novaluron (82.21%). The lowest efficacy was found in bifenthrin (73.42%) and acephate (72.62%) against *P. xylostella* larvae (Table 2).

### Management of *P. xylostella* through novel insecticides in cauliflower during rabi, 2018-19

#### Efficacy of insecticides against larvae of *P. xylostella*

##### At 3 days after application of insecticides

Before application of insecticides the pre-treatment count of larvae per plant were in the range of 5.87 to 6.67. The treatments spinetoram, tolfenpyrad and cyantraniliprole were found to be more superior in control of *P. xylostella* with the mean per cent reduction of 94.45, 94.44 and 94.31 respectively, which were on par with each other. These treatments were followed by chlorantraniliprole (84.65%), spinosad (83.83%), emamectin benzoate (80.43%) and novaluron (75.53%) with significant difference. The least effective treatments were bifenthrin (72.45%) and acephate (68.37%) (Table 3).

##### At 5 days after application of insecticides

The observations at day 5 revealed that the efficacy against *P. xylostella* larvae was the highest in tolfenpyrad (91.31%) followed by cyantraniliprole

(90.30%) and spinetoram (90.26%) without any significant difference among them. The next best treatments were spinosad (84.35%), chlorantraniliprole (83.27%) and emamectin benzoate (80.62%) followed by novaluron (75.53%), bifenthrin (66.20%) and acephate (64.18%) respectively (Table 3).

##### At 7 days after application of insecticides

The data at day 7 clearly revealed that the insecticidal treatments cyantraniliprole, tolfenpyrad and spinetoram were highly effective against *P. xylostella* larvae with mean per cent reduction of 90.96, 90.71 and 90.10, respectively. The spinosad, chlorantraniliprole and emamectin benzoate were the next best treatments with 85.58, 83.52 and 80.43 per cent control of *P. xylostella* larvae. The low potency of control was observed in novaluron, bifenthrin and acephate representing the per cent *P. xylostella* larvae control of 78.41, 66.40 and 65.90 respectively.

The mean efficacy of insecticides against *P. xylostella* was the highest with insecticide tolfenpyrad (92.15%), cyantraniliprole (91.85%) and spinetoram (91.60%) followed by spinosad (84.58%), chlorantraniliprole (83.81%), emamectin benzoate (80.49%) and novaluron (77.88%) respectively. The lowest efficacy against *P. xylostella* larvae was observed in bifenthrin (68.35%) and acephate (66.15%) (Table 3).

**Table 3:** Efficacy of certain insecticides against *P. xylostella* larval population in cauliflower during rabi, 2018-19

Sl. No.	Treatment	Dosage	PTC (No. of larvae per plant)	Per cent efficacy over control			
				3 DAA	5 DAA	7 DAA	Mean
T1	Bifenthrin 10 EC	1.5 mL/L	6.67	72.45 (58.42) <sup>cd</sup>	66.20 (54.49) <sup>d</sup>	66.40 (54.60) <sup>e</sup>	68.35 (55.79) <sup>e</sup>
T2	Emamectin Benzoate 5 SG	0.4 g/L	5.93	80.43 (63.82) <sup>c</sup>	80.62 (63.88) <sup>bc</sup>	80.43 (63.90) <sup>cd</sup>	80.49 (63.79) <sup>cd</sup>
T3	Tolfenpyrad 15 EC	2 mL/L	6.40	94.44 (76.48) <sup>a</sup>	91.31 (72.91) <sup>a</sup>	90.71 (72.27) <sup>a</sup>	92.15 (73.82) <sup>a</sup>
T4	Cyantraniliprole 10.26 OD	0.3 mL/L	6.33	94.31 (76.51) <sup>a</sup>	90.30 (72.41) <sup>a</sup>	90.96 (72.51) <sup>a</sup>	91.85 (73.52) <sup>a</sup>
T5	Spinosad 45 SC	0.3 mL/L	6.07	83.83 (66.30) <sup>b</sup>	84.35 (66.72) <sup>b</sup>	85.58 (67.72) <sup>b</sup>	84.58 (66.89) <sup>b</sup>
T6	Chlorantraniliprole 18.5 SC	0.3 mL/L	6.00	84.65 (67.12) <sup>b</sup>	83.27 (66.08) <sup>b</sup>	83.52 (66.06) <sup>bc</sup>	83.81 (66.28) <sup>bc</sup>
T7	Spinetoram 11.7 SC	1 mL/L	5.87	94.45 (76.42) <sup>a</sup>	90.26 (71.88) <sup>a</sup>	90.10 (71.72) <sup>a</sup>	91.60 (73.28) <sup>a</sup>
T8	Novaluron 10 EC	1 mL/L	6.13	79.71 (63.36) <sup>c</sup>	75.53 (60.39) <sup>c</sup>	78.41 (62.32) <sup>d</sup>	77.88 (61.96) <sup>d</sup>
T9	Acephate 75 SP	1 g/L	6.47	68.37 (56.01) <sup>d</sup>	64.18 (53.27) <sup>d</sup>	65.90 (54.28) <sup>e</sup>	66.15 (54.43) <sup>e</sup>
T10	Untreated control		6.13	—	—	—	—
F Test of Significance @ df=9				Sig.***	Sig.***	Sig.***	Sig.***
CD (P = 0.05)				4.94	4.81	2.65	2.06
SE(M)				1.66	1.62	0.90	0.70

PTC – Pre Treatment Count, DAA – Days After Application; Values are the means of three replication; Values in the parentheses are arcsine transformed values; Means followed by different letters are significantly different by DMRT ( $P \leq 0.05$ , LSD); \*\*\*= $P \leq 0.005$ .



## Cumulative efficacy of novel insecticides against *P. xylostella* larvae and leaf damage in rabi, 2017-18 and 2018-19

### Efficacy of insecticides against larvae of *P. xylostella*

The cumulative pre-treatment counts of *P. xylostella* larvae per plant in rabi, 2017-18 and 2018-19 were in the range of 5.53 to 6.07. At 3 DAA, the tolfenpyrad, spinetoram and cyantraniliprole were found to be the most effective to reduce the population of *P. xylostella* larvae with per cent control efficacy of 92.87, 92.76 and 92.09 followed by chlorantraniliprole and spinosad with 84.55 and 82.59 per cent control. However, the next best insecticides were emamectin benzoate and novaluron showing 80.74 and 79.16 per cent control, respectively. The least effective insecticides were acephate and bifenthrin with 70.64 and 75.43 per cent potency over control (Table 4 and Fig. 1).

At 5 DAA, the, tolfenpyrad (91.23%) cyantraniliprole (90.95%) and spinetoram (90.40%) were best against *P. xylostella* larvae followed by chlorantraniliprole (84.45%) and spinosad (83.55%). The emamectin benzoate (81.08%) and novaluron (79.67%) were next best insecticides. The least effective insecticides were bifenthrin (69.30%) and acephate (68.81%). Similar trend was observed at 7 DAA, the per cent

control of 91.06, 90.92 and 90.72 was observed in spinetoram, cyantraniliprole and tolfenpyrad which were superior over other and on par with each other. The next best insecticides were spinosad (86.00%), chlorantraniliprole (84.65%) followed by emamectin benzoate (81.35%) and novaluron (80.31%) while, the lowest efficacy was recorded in the acephate (69.26%) and bifenthrin (69.74%) (Table 4).

The mean per cent *P. xylostella* larval control was highest in tolfenpyrad (91.61%), spinetoram (91.41%) and cyantraniliprole (91.32%) followed by chlorantraniliprole (84.55%), spinosad (84.05%), emamectin benzoate (81.06%) and novaluron (79.71%). The bifenthrin (71.49%) and acephate (69.57%) shown least per cent efficacy mentioned in Table 4.

The results on the efficacy of insecticides against the control of *P. xylostella* larvae and on per cent leaf damage over control were documented. In both the seasons, the most effective insecticide formulations were tolfenpyrad 15 EC @ 2 mL L<sup>-1</sup>, cyantraniliprole @ 0.3 mL L<sup>-1</sup> and spinetoram 11.7 SC @ 1 mL L<sup>-1</sup>. The present experimental results are in similarity with findings of Bajpai *et al.* (2014) who reported that the highest control was recorded in the tolfenpyrad @ 100, 125 and 150 g a.i. ha<sup>-1</sup> compare to the other insecticides cartap hydrochloride and chlorfenapyr. Similarly, Kodandaram *et al.* (2017) also observed

**Table 4:** Cumulative efficacy of certain insecticides against by *P. xylostella* larval population in cauliflower during rabi, 2017-18 and 2018-19

Sl. No.	Treatment	Dosage	PTC (No. of larvae per plant)	Per cent efficacy over control			
				3 DAA	5 DAA	7 DAA	Mean
T1	Bifenthrin 10 EC	1.5 mL/L	5.90	75.43 (60.30) <sup>cd</sup>	69.30 (56.35) <sup>c</sup>	69.74 (56.64) <sup>d</sup>	71.49 (57.75) <sup>d</sup>
T2	Emamectin benzoate 5 SG	0.4 g/L	5.67	80.74 (63.98) <sup>bc</sup>	81.08 (64.26) <sup>b</sup>	81.35 (64.49) <sup>c</sup>	81.06 (64.20) <sup>c</sup>
T3	Tolfenpyrad 15 EC	2 mL/L	6.57	92.87 (74.58) <sup>a</sup>	91.23 (72.80) <sup>a</sup>	90.72 (72.26) <sup>a</sup>	91.61 (73.18) <sup>a</sup>
T4	Cyantraniliprole 10.26 OD	0.3 mL/L	5.83	92.09 (73.72) <sup>a</sup>	90.95 (72.76) <sup>a</sup>	90.92 (72.46) <sup>a</sup>	91.32 (72.87) <sup>a</sup>
T5	Spinosad 45 SC	0.3 mL/L	6.07	82.59 (65.47) <sup>b</sup>	83.55 (66.11) <sup>b</sup>	86.00 (68.05) <sup>b</sup>	84.05 (66.48) <sup>b</sup>
T6	Chlorantraniliprole 18.5 SC	0.3 mL/L	5.77	84.55 (66.89) <sup>b</sup>	84.45 (66.86) <sup>b</sup>	84.65 (66.97) <sup>b</sup>	84.55 (66.85) <sup>b</sup>
T7	Spinetoram 11.7 SC	1 mL/L	5.53	92.76 (74.48) <sup>a</sup>	90.40 (72.00) <sup>a</sup>	91.06 (72.64) <sup>a</sup>	91.41 (72.98) <sup>a</sup>
T8	Novaluron 10 EC	1 mL/L	5.57	79.16 (62.94) <sup>bc</sup>	79.67 (63.22) <sup>b</sup>	80.31 (63.67) <sup>c</sup>	79.71 (63.23) <sup>c</sup>
T9	Acephate 75 SP	1 g/L	5.70	70.64 (57.25) <sup>d</sup>	68.81 (56.08) <sup>c</sup>	69.26 (56.33) <sup>d</sup>	69.57 (56.52) <sup>d</sup>
T10	Untreated Control		5.77	—	—	—	—
F Test of Significance @ df=9				Sig.***	Sig.***	Sig.***	Sig.***
CD (P = 0.05)				3.96	3.05	2.11	1.68
SE(M)				1.33	1.03	0.71	0.56

PTC – Pre Treatment Count, DAA – Days After Application; Values are the means of three replication; Values in the parentheses are arcsine transformed values; Means followed by different letters are significantly different by DMRT ( $P \leq 0.05$ , LSD); \*\*\*= $P \leq 0.005$ .

that the cyantraniliprole @ 60, 75 and 90 g a.i. ha<sup>-1</sup> was superior in control of the *P. xylostella* and other sucking pest in contrast to the emamectin benzoate, spinosad, chlorantraniliprole and other conventional insecticides. Huang *et al.* (2011) described that spinetoram was potent to control more than 90 per cent of *P. xylostella* at 3 Days after imposition of treatments @ 13 to 38 g a.i. ha<sup>-1</sup> doses, it was comparatively superior to indoxacarb @ 36 g a.i. ha<sup>-1</sup> and chlorfenapyr 60 g a.i. ha<sup>-1</sup>.

The present investigations also revealed that the second best insecticide formulations to control *P. xylostella* were spinosad 45 SC @ 0.3 mL L<sup>-1</sup> and chlorantraniliprole 18.5 SC @ 0.3 mL L<sup>-1</sup> and the least effective treatments were bifenthrin @ 1.5 mL L<sup>-1</sup> and acephate @ 1 g L<sup>-1</sup>. As per report of Dotsara *et al.* (2017), the spinosad @ 45 SC @ 0.5mL L<sup>-1</sup> was the best treatment among flubendiamide 48 SC @ 0.3 ml/L and chlorantraniliprole 18.5 SC @ 0.3 g L<sup>-1</sup> on cauliflower. Experimental findings of Sawant and Patil (2018) confirms the effectiveness of chlorantraniliprole @ 10 g a.i. ha<sup>-1</sup> followed by spinosad @ 17.5 g a.i. ha<sup>-1</sup> over indoxacarb, emamectin benzoate, diafenthiuron and also reported that was the formulation of bifenthrin @ 50 g a.i. ha<sup>-1</sup> recorded least control of *P. xylostella*.

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# Study on Drying Characteristics of *Simarouba glauca* Leaves

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## ABSTRACT

*Simarouba glauca* (Family: *Simaroubaceae*) leaves possess analgesic, antibacterial, anticancer, antifungal, antimicrobial, antioxidant, antiviral, tonic and vermifuge properties. Solar and biomass drying in a closed system could be an alternative to overcome the disadvantages of conventional methods viz. sun, shade and tray drying. Hybrid drying using solar and biomass energy has advantages over efficiency, losses, quality control and time. *Simarouba glauca* leaves were dried with two different renewable energies i.e. solar, biomass and combination of both. Drying rate along with moisture content and properties of drying, ambient and exhaust air were recorded at definite time intervals for *Simarouba* leaves. Total drying time of 34 h, 18 h and 20 h and average drying air temperature of 34.8°C, 45.8°C and 43.1°C was observed for solar, biomass and hybrid drying, respectively. The reduction in time of hybrid drying was observed 41% as compared to solar drying. About 33 % less fuel (Briquettes) requirement resulted for hybrid drying than biomass drying. Colour values were retained well with low-temperature drying. Total phenols and Total flavonoids were affected by drying air temperature, time of solar radiation exposure. Hybrid drying would be more effective than solar and biomass for drying of *Simarouba* leaves.

## HIGHLIGHTS

- Importance of *Simarouba glauca* leaves and its drying.
- Different conventional method of drying.
- Drying characteristics and phytochemical properties of *Simarouba glauca* leaves.

**Keywords:** *Simarouba glauca*, solar drying, biomass drying, hybrid drying, drying characteristics

*Simarouba glauca* - a 'Paradise Tree' or 'Laxmitaru', belongs to the family *Simaroubaceae*. *Glauca* word is derived from the Greek word 'Glaukos', which means covered with bloom, which refers to the bluish-green foliage. In India, it was introduced in Maharashtra and Bangalore in the years 1966 and 1986, respectively for the research purpose. Now the cultivation has spread to Gujarat, Tamilnadu, Maharashtra, Karnataka and Andhra Pradesh. The fruit, leaf, pulp and seed of *Simarouba glauca* is known to possess medicinal properties such as analgesic, antimicrobial, antiviral, astringent, emmenagogue, stomachic, tonic and vermifuge (Joshi and Joshi 2002). *Simarouba* bark and leaf extracts are well known for their pharmacological properties; anti-cancerous, anti dysenteric, antihelminthic,

antiparasitic, antipyretic and haemostatic properties (Varghese *et al.* 2016). *Simarouba glauca* leaves are pinnately compound with 3-21 oblong leaflets, notched or smooth at apex, alternate, even, bluish oily green in colour of 20 to 50 cm length (Osagie-Eweka *et al.* 2016).

*Simarouba* leaf extracts reported for the presence of alkaloids, flavonoids, phenolic compounds and saponins (Kumar *et al.* 2016; Sajeeda *et al.* 2019; Santhosh *et al.* 2016; Sharanya *et al.* 2016)

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and Varghese *et al.* 2016). Simarouba leaves were reported with properties such as antibacterial (Jangale *et al.* 2012), anticancer (Kumar *et al.* 2016), antifungal (Mikawlawng *et al.* 2014), antimicrobial (Rajurkar 2011) and antioxidant (Osagie-Eweka *et al.* 2016) properties. The study of the *Simarouba glauca* leaves, therefore, opened up a whole new window for scientists and researchers.

Recently, many studies have been conducted on the investigation of renewable-based drying behaviour of different plant leaves using different drying methods. Doymaz *et al.* (2006) studied the drying characteristics of dill and parsley leaves. Bahloul *et al.* (2009) studied the convective solar drying of olive leaves (*Olea europaea* L.). Akpinar (2010) investigated thin-layer drying characteristics of mint leaves in solar cabinet drying with forced convection and open sun drying with natural convection. Arabhosseini *et al.* (2011) investigated the effect of drying on the colour change in tarragon (*Artemisia dracuncululus* L.) leaves. Kenghe *et al.* (2015) evaluated the quality characteristic of curry leaves dried using different drying methods *viz.* sun drying, shade drying and tray drying. Hot air oven and shade drying methods were observed for drying of Simarouba leaves with a small quantity of product suitable to lab-scale (Gurupriya *et al.* 2017; John *et al.* 2016; Manasa *et al.* 2019; Mikawlawng *et al.* 2014 and Santhosh *et al.* 2015).

The present climatological and economic situation of the country demands an eco-friendly and cheap source of energy for drying. In non-electrified rural areas, drying of agricultural products using solar or biomass as energy sources is preferred due to the unavailability of electricity and expensive fossil fuels. Limitations of shade drying are quantitative losses of produce by animals, birds and rodents and degradation in quality due to contamination by dirt, dust or debris. Also, chances of insect infestation and growth of microorganisms due to non-uniform drying are more in shade drying. Therefore, solar or biomass drying in a closed system can be the alternative technique to obtain quality dried products. The hybrid dryer integrated with solar and biomass energy gives advantages in enhancing drying efficiency, reduction in losses, control over quality and reduction in operational time. To facilitate the drying operation, the experiment was carried out with the help of solar-cum-biomass

energy hybrid dryer (unpublished) consisting of a solar cabinet, cylindrical drying chamber and biomass energy heat exchanger developed at ICAR - AICRP on PHET, UAS-Bangalore.

## MATERIALS AND METHODS

### Materials

Well matured, diseases free, fresh and healthy leaves of Simarouba were harvested from trees of UAS-B, GKVK Campus, Bengaluru, washed and drained well. All the chemicals and reagents used to measure parameters in the study were of analytical grade.

### Properties of Fresh Simarouba Leaves

Properties such as moisture content (% w.b. and % d.b.), bulk density, true density, surface area and size were measured in replications of three and average values were reported. The moisture content was determined by measuring the weight loss of the sample in a moisture box by desiccation in an oven maintained at 105 °C until constant weight (AOAC, 2005). Bulk density and true density were calculated from the mass of bulk material divided by volume containing the mass and the true volume of the bulk material using the toluene (C<sub>7</sub>H<sub>8</sub>) displacement method, respectively (Nayak *et al.* 2016). The surface area of the leaves was measured by tracing a leaf area on Cartesian graph paper (King 2015). Size in terms of length, width and thickness were measured using digital vernier calliper (Model: CD-6BS-Mitutoyo Corporation, Japan) with an accuracy of ± 0.01 mm.

### Studies on Drying Characteristics of Simarouba Leaves

About 7.5 kg (half load capacity) of fresh leaves were loaded in the dryer for solar drying, biomass drying and solar-cum-biomass energy hybrid drying methods. For solar drying, sun-shine exposure time was from 09:00 a.m. to 07:00 p.m. a day for the record, whereas in biomass drying, under the shaded conditions, continuous burning of briquettes was used as a heat source to heat drying air. Drying data were recorded continuously in biomass drying. In the hybrid drying method, both solar and biomass energy were used to dry the Simarouba leaves. The drying chamber loaded with fresh leaves

was exposed to solar radiation during sun-shine hours (09:00 a.m. to 05:00 p.m.). The heat obtained through biomass burning was utilised during non-shiny hours like early morning (07:00 a.m. to 09:00 a.m.), late evening (05:00 p.m. to 03:00 a.m.) and cloudy weather. All the drying data were recorded at an interval of 2 h.

Sampling was carried out with 500 g fresh leaves samples packed in the plastic mesh bags/net bags placed inside the drying chamber at different zones. These bags were withdrawn periodically for measurement of moisture loss. Drying data such as initial, intermediate and final moisture content of the drying material, psychrometric data (temperature and relative humidity) of drying air, exhaust air and ambient air were measured at regular intervals with drying time. Drying rate (DR) and moisture ratio (MR) were calculated and plotted against time along with Moisture content (% d.b.) v/s time. Total colour difference ( $\Delta E^*$ ), total phenols and total flavonoids were analysed for different drying methods.

### Drying rate

Drying rate (kg of water removed/kg of dry matter.h) was determined based on the quantity of water evaporated from the samples and the drying time. The quantity of water evaporated from the product during drying was calculated as the difference between the initial and final weight of the sample (Kamble and Dombale 2015).

Drying rate (DR) =

$$\frac{\Delta M, \text{Quantity of water evaporated from product (kg)}}{\Delta t, \text{Time required to dry the product (h)}} \dots (1)$$

### Moisture ratio

Moisture ratio (MR), a drying characteristics parameter was calculated by the following mathematical expression described by Kamble and Dombale (2015).

$$MR = \frac{M - M_e}{M_0 - M_e} \dots (2)$$

Where,  $M$  = Moisture content at a given time (% d.b.);  $M_e$  = Equilibrium moisture content (% d.b.);  $M_0$  = Initial moisture content (% d.b.).

Effect of different drying methods on drying time, drying rate and quality parameters of dried product were analysed using One-way Analysis of Variance (ANOVA) at 5 % significance level and mean data are presented.

## Quality Characteristics

### Colour

A spectrophotometer (Make: Konica Minolta Instrument, Osaka, Japan; Model - CM5) was used to measure tristimulus colour values. It was calibrated with black and white standard plates before colour analysis. The colour of the sample was measured in  $L^* a^* b^*$  coordinate system; where  $L^*$  indicates lightness;  $a^*$  value indicates greenness (-) or redness (+) and  $b^*$  value indicates blueness (-) or yellowness (+) of the sample. The difference in  $L^*$ ,  $a^*$ ,  $b^*$  values of fresh and dried leaves were used to calculate colour change or total colour difference ( $\Delta E^*$ ) using the Hunter-Scotfield equation as described by Spada *et al.* (2012). The observations were replicated thrice and average data were reported.

$$\Delta E^* = \left[ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{\frac{1}{2}}$$

### Total Phenols

Total phenols (mg Gallic Acid Equivalents/100 g) were measured as described by Singleton and Rossi (1965) and presented on dry basis. About 1 g of sample was crushed in pestle and mortar and the extract was obtained using 80 % methanol (20 ml). The extraction was repeated twice and the volume was made up to 50 ml from the pooled extracts. One ml of extract was taken and diluted with 80 % methanol. About 0.2 ml of Folin Ciocalteu Reagent (FCR) was added to 0.5 ml of diluted extract followed by the addition of 3.3 ml of distilled water and the solution was thoroughly mixed. After 2 minutes, 1 ml of sodium carbonate solution was added, thoroughly mixed in test tubes and incubated at room temperature for 30 minutes. The intensity of blue colour was measured in a spectrophotometer (Make: Systronics, Model: UV-VIS 118) at 700 nm. A standard curve for phenols was prepared using gallic acid as a standard. An average of three replications were reported.



Total phenols (mg Gallic Acid equivalents/100 g) =

$$\frac{\text{OD}_{700} \times \text{Std. value } (\mu\text{g}/\text{OD}) \times \text{Total extract Vol.} \times 100 \times \text{Dilution factor}}{\text{Assay volume} \times \text{Weight of sample (g)} \times 1000}$$

### Total Flavonoids

Total flavonoids (mg Quercetin equivalents/100 g) of Simarouba leaves were estimated using quercetin as a standard and presented on dry basis (Chun *et al.* 2003). Methanolic extracts (1 mL) was obtained and diluted with 80 % methanol for the required volume. The diluted extract was filled in a test tube and 0.3 mL of 5 % NaNO<sub>2</sub> was added twice at an interval of 2 minutes. Similarly, 3.4 mL of 4 N NaOH was added after 2 minutes and the mixture was incubated at room temperature for 10 minutes. The brick red colour was measured with the three replications at 510 nm in a spectrophotometer.

Total flavonoids (mg Quercetin equivalents/100 g) =

$$\frac{\text{OD}_{510} \times \text{Std. value (mg}/\text{OD}) \times \text{Total extract Vol.} \times 100 \times \text{Dilution factor}}{\text{Assay volume} \times \text{Weight of sample (g)}}$$

## RESULTS AND DISCUSSION

### Properties of fresh Simarouba leaves

The properties of fresh Simarouba leaves like moisture content, bulk density, true density, leaf surface area, total phenols, total flavonoids, size and colour were measured. The fresh leaves contained 64.25 % of moisture (179.79 % d.b.) with bulk density and true density of 72.75 kg/m<sup>3</sup> and 1048.98 kg/m<sup>3</sup>, respectively. The average dimensions (size) of leaves recorded were 8.72 cm in length, 2.77 cm in width and 0.045 cm in thickness and the mean surface area of the well-matured fresh leaf was 20.26 cm<sup>2</sup>. Fresh Simarouba leaves contained 377.90 mg GAE/g of total phenols (dry basis) and 253.61 mg Quercetin/g of total flavonoids (dry basis). L\*, a\* and b\* values of fresh Simarouba leaves were found to be 39.23, -5.43 and 9.67, respectively, indicating the dark green colour of leaves.

### Drying characteristics of Simarouba leaves

The drying characteristics; Drying rate (DR); kg/

kg<sub>d</sub>.h along with Moisture Content (% d.b.), and Moisture ratio (MR) for Simarouba leaves were observed for different drying methods *viz.* solar drying (Fig. 1), biomass drying (Fig. 3) and solar-cum-biomass (Hybrid) drying (Fig. 5) at 2 h of the time interval.

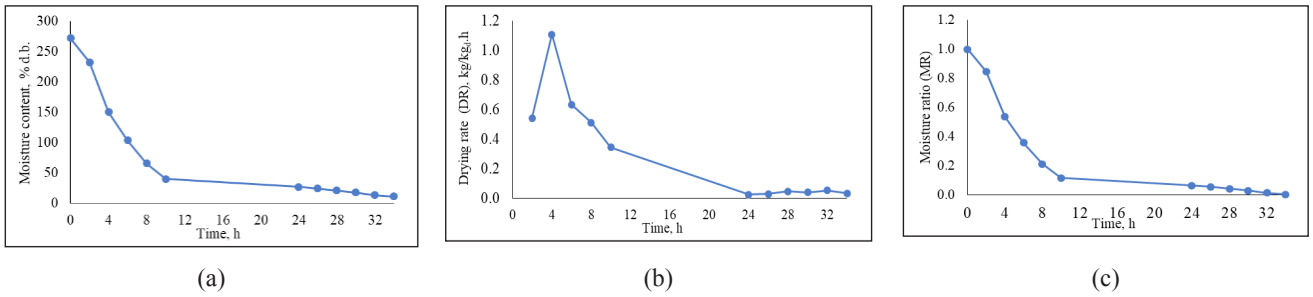
### Solar drying

A batch of 7.5 kg sample was dried under bright sun-shine from 09:00 a.m. to 07:00 p.m. and moisture evaporated with time was recorded. The total drying time was 34h with 14h non-recording time. Drying rate was observed in decreasing order with a duration of drying and even on consecutive days. A maximum temperature (41.1 °C) and the minimum relative humidity of drying air (19.5 %) were observed at 01:00 p.m. noon as presented in Fig. 2. An average temperature of drying air (34.8 °C) was found 5.9 °C higher than the ambient air temperature (28.9 °C). Average temperature difference between drying air and exhaust air was 3.2 °C. The temperature difference of 5 °C to 26 °C between ambient air and drying air during solar drying of different products was reported by Arjoo *et al.* (2017); Padmapani *et al.* (2019); Prasad *et al.* (2006) and Sengar *et al.* (2018). Akpınar (2010) observed the temperature of ambient air, drying air and exhaust air in the same manner for solar drying.

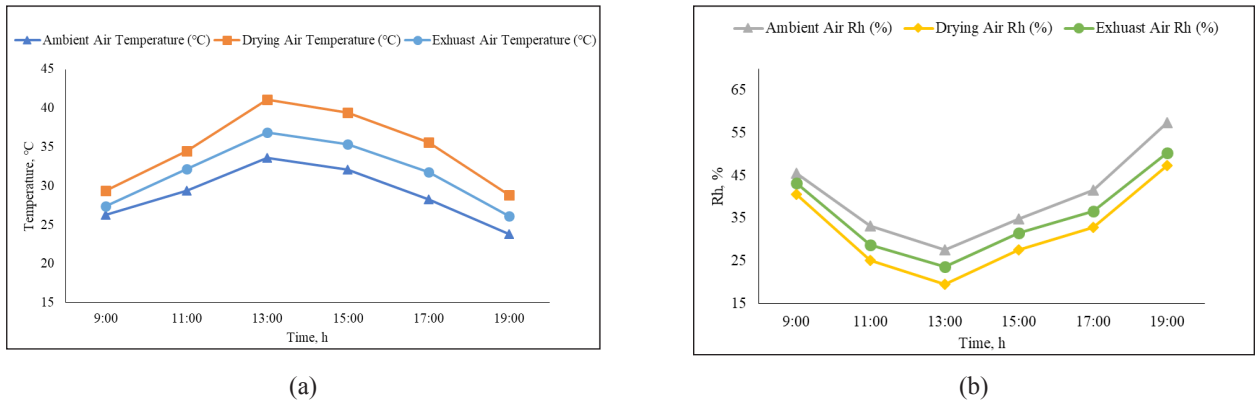
### Biomass drying

During biomass drying, briquettes were fed continuously at a feed rate of 2 kg/h to raise the temperature of drying air for 18 h of drying. The drying rate was observed in decreasing order at almost constant temperature after the initial 30 minutes of the lag period to attain the average temperature (45.8 °C) of drying air. As per Fig. 4, maximum temperature and minimum humidity of ambient air were recorded as 34.4 °C and 22.5 %, respectively. The average temperature difference between drying air and exhaust air was 5.6 °C, which decreased with time. Geramitchioski *et al.* (2011) reported that about 60 ° of drying air temperature could be obtained by combusting 4 kg wood briquettes per hour. The maximum temperature of drying air could be raised up to 60 °C by using a biomass burner with a thermal backup unit for gas to a gas heat exchanger (Yassen *et al.* 2013).

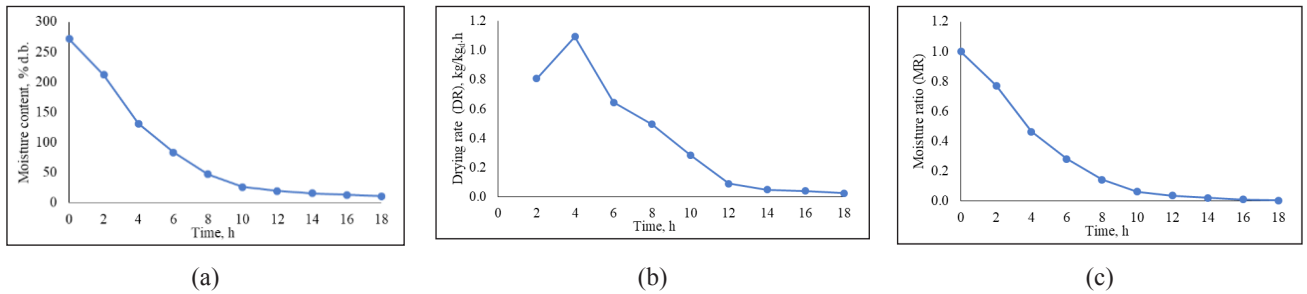




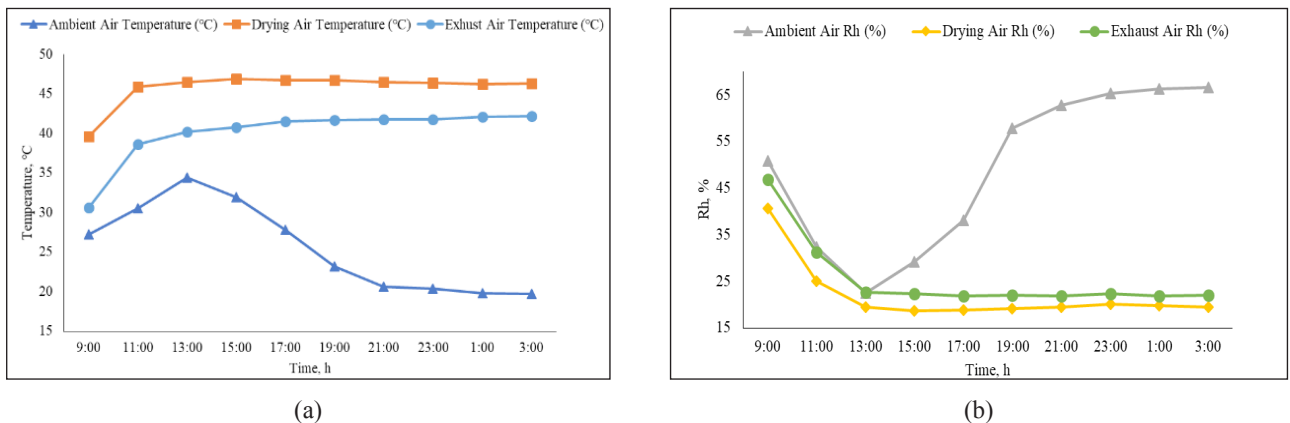
**Fig. 1:** Drying characteristics of simarouba leaves for solar drying; **(a)** Moisture content (% d.b.) v/s Time (h); **(b)** Drying rate (DR) v/s Time (h); **(c)** Moisture ratio (MR) v/s Time (h)



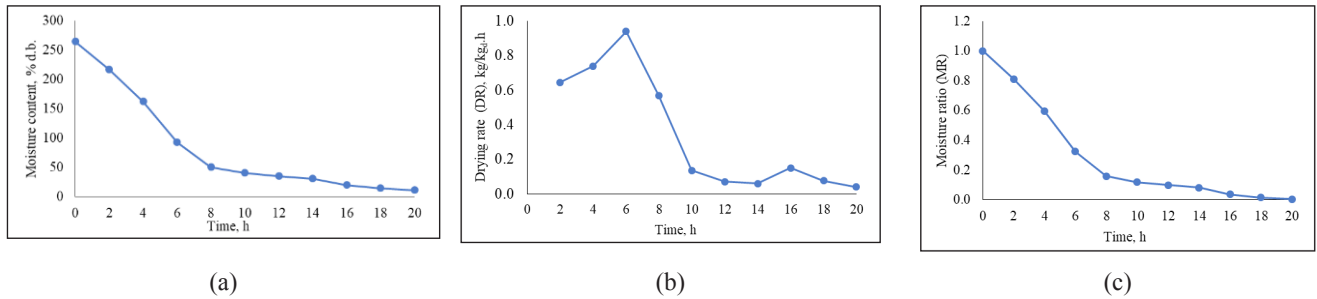
**Fig. 2:** Psychrometric properties of air during solar drying; **(a)** Temperature (°C) profile; **(b)** Relative humidity, RH (%) profile



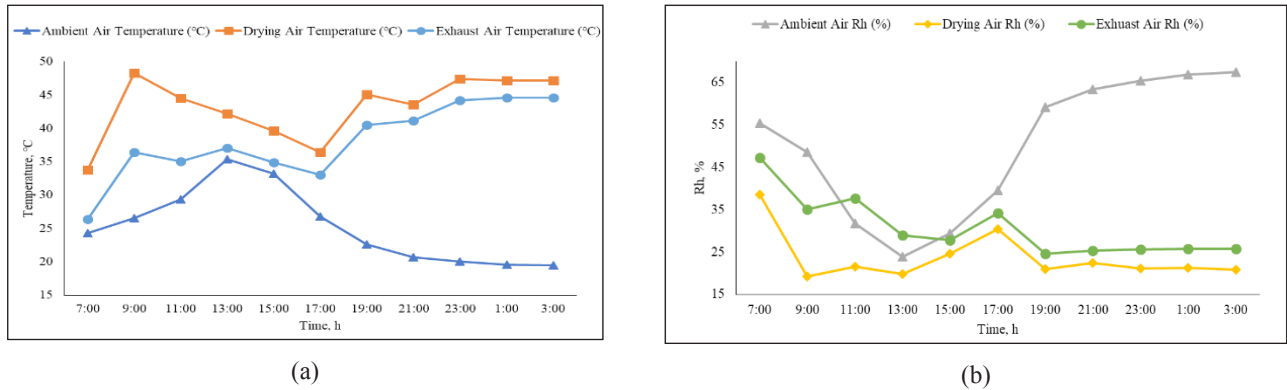
**Fig. 3:** Drying characteristics of simarouba leaves for biomass drying; **(a)** Moisture content (% d.b.) v/s Time (h); **(b)** Drying rate (DR) v/s Time (h); **(c)** Moisture ratio (MR) v/s Time (h)



**Fig. 4:** Psychrometric properties of air during biomass drying; **(a)** Temperature (°C) profile; **(b)** Relative humidity, RH (%) profile



**Fig. 5:** Drying characteristics of simarouba leaves for Hybrid drying; **(a)** Moisture content (% d.b.) v/s Time (h); **(b)** Drying rate (DR) v/s Time (h); **(c)** Moisture ratio (MR) v/s Time (h)



**Fig. 6:** Psychrometric properties of air during hybrid drying; **(a)** Temperature (°C) profile; **(b)** Relative humidity, RH (%) profile

### Solar-cum-biomass (Hybrid) drying

To overcome the limitations of solar drying and biomass drying, continuous drying was ensured between 07:00 a.m. to 03:00 a.m. (20h). The average temperature of drying air was observed 41.1°C, 40.7 °C and 46.1 °C during biomass, solar and biomass energy source, respectively. An average temperature (43.2 °C) and average relative humidity (23.7 %) was observed for hybrid drying (Fig. 6). The average temperature gradient between drying air and ambient air was observed to be 17.9 °C; whereas 26.4 % of gradient was observed in average relative humidity. Approximately, 30.7 % of total moisture was removed in 8 h by solar drying and the remaining 69.3 % was removed in 12 h with the help of biomass energy. Bena and Fuller (2002) reported that 47 % of moisture would be removed by solar energy. Okoroigwe *et al.* (2013) reported that the drying with the combination of solar and biomass heat for day and night would improve the dryer efficiency.

### Drying parameters for bulk drying of simarouba leaves

As presented in Table 1, drying studies on bulk

drying of Simarouba leaves resulted in the final moisture content of leaves being reduced from 64 % to ~10 % during a drying period of 34 h (Solar drying), 18 h (Biomass drying) and 20 h (Hybrid drying). Statistically, the average temperatures were significantly different for each drying. The average drying temperature for solar, biomass and hybrid drying was 34.8, 45.8 and 43.2 °C, respectively. The drying time was reduced by 41 % in hybrid drying as compared to solar drying which is highly significant. Kenghe *et al.* (2015) reported that tray drying reduced 27 % of the time for curry leaves as compared to shade and sun drying.

Yahya (2016) reported the solar-assisted heat pump dryer for red chillies saved 82 % drying time as compared to open sun drying. The drying rate in hybrid drying (0.2253 kg/kg<sub>d</sub>.h) was lower than biomass drying and higher than solar drying. The rate of drying for yam chips was recorded as 0.0142 kg/h for hybrid drying and 0.00732 kg/h for solar drying (Okoroigwe *et al.* 2013). Yahya (2016) observed a 1.57 kg/h of drying rate in the case of yam chips drying in a solar-assisted heat pump dryer. The final moisture content of the product for hybrid drying was statistically the same as for solar

**Table 1:** Drying parameters of simarouba leaves under different drying methods

Treatment	Drying air temperature (°C)	Drying Time (h)	Drying Rate (kg/kg <sub>d</sub> .h)	Final MC (% w.b.)
Solar Drying	34.8 <sup>c</sup> ± 1.36	34 <sup>a</sup> ± 2	0.1319 <sup>c</sup> ± 0.003	10.41 <sup>a</sup> ± 0.42
Biomass Drying	45.8 <sup>a</sup> ± 1.38	18 <sup>b</sup> ± 5	0.2536 <sup>b</sup> ± 0.007	9.79 <sup>b</sup> ± 0.33
Hybrid drying	43.1 <sup>b</sup> ± 1.13	20 <sup>b</sup> ± 2	0.2253 <sup>c</sup> ± 0.005	10.70 <sup>a</sup> ± 0.43
SEM	0.58	1.26	0.002	0.18
CD @5 %	1.78	3.90	0.007	0.55
C.V. (%)	3.14	11.785	2.540	3.86

**Table 2:** Quality parameters of Simarouba leaves dried under different methods

Treatment	Colour difference (ΔE*)	Total phenols (mg GAE/g)	Total flavonoids (mg Quercetin/g)
Solar Drying	29.09 <sup>a</sup> ± 1.22	254.93 <sup>a</sup> ± 34.96	152.92 <sup>b</sup> ± 23.80
Biomass Drying	24.13 <sup>c</sup> ± 0.51	275.28 <sup>a</sup> ± 20.88	193.09 <sup>a</sup> ± 13.20
Hybrid drying	27.02 <sup>b</sup> ± 0.97	282.18 <sup>a</sup> ± 25.57	<b>194.65<sup>a</sup> ± 10.83</b>
SEM	0.42	12.68	7.56
CD @5 %	1.30	39.12	23.31
C.V. (%)	3.53	10.48	9.38

drying. The final moisture content of Simarouba leaves was reported to be approximately 10 % under shade drying (Manasa *et al.*, 2019). The total quantity of briquettes used to produce dried leaves was 24 kg and 36 kg in hybrid drying and biomass energy drying, respectively. About 33 % of the fuel requirement in the form of biomass briquettes was cut down in hybrid drying as compared to biomass drying.

#### Effect of different drying methods on colour, total phenols and total flavonoids

The difference in the colour value compared to fresh Simarouba leaves was calculated and presented in Table 2 with respect to ΔE\* value. A higher value of L\* indicated the lighter colour of dried leaves as compared to fresh leaves. Yellowing of leaves was observed during drying for all methods. In all drying methods, the green colour was retained in dried leaves as similar to the green colour of fresh leaves with slight lightness. However, the overall colour difference (ΔE\*) was significantly different for each treatment with the highest change in solar drying and lowest change in biomass drying.

Total phenols and total flavonoids on the dry basis ranged from 254.93 to 282.18 mg GAE/g and 152.92 to 194.65 mg Quercetin/g, respectively after drying of Simarouba leaves. Totals phenols were

significantly the same for all the treatments, whereas total flavonoids were observed significantly different for solar drying as compared to biomass and hybrid drying. Higher retention in phytochemical values may be due to lower photo-oxidation and lower temperature. The photo-oxidation in solar drying and the higher temperature in biomass drying resulted in lower retention of phytochemical properties in dry leaves as well as it affected colour values, also. Simarouba leaves dried under the hybrid drying method retained about 74.65 % and 76.84 % of total phenols and total flavonoids, respectively, as compared to fresh leaves. Total phenols of 102.3 ± 0.0027 mg/g GAE was reported for Simarouba leaves by Gurupriya *et al.* (2017). Total phenols and total flavonoids of fresh Simarouba leaves were reported to be 151.07 ± 11.05 and 288.99 ± 27.08 mg GAE/g, respectively, whereas for shade dried leaves, these values were reported as 88.03 ± 12.00 and 102.84 ± 4.00 mg Quercetin/g, respectively (Manasa *et al.* 2019).

#### CONCLUSION

A study on the bulk drying of *Simarouba glauca* leaves reveals that the drying rate decreases with drying time in all the methods due to a decrease in the amount of moisture present in the product. Drying time for solar (34 h), biomass (18 h) and



hybrid drying (20 h) was affected by the average drying air temperature (34.8, 45.8 and 43.1 °C, respectively). A higher temperature of drying air lowers the drying time. The amount of fuel (Briquettes) used was 33 % less in hybrid drying than biomass drying with only a 10 % change in drying time. Colour values, in contrast to drying time, were retained well with lower drying air temperature and less photo-oxidation. Total phenols and Total flavonoids were affected by drying air temperature and time of solar radiation exposure. The phytochemical values were retained well in hybrid drying. The reduction in time of hybrid drying was observed at 41 % as compared to solar drying. Thus, hybrid drying would be the effective treatment to dry the leaves of *Simarouba* in bulk with renewable energy sources, which overcomes the disadvantages of solar and biomass drying.

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# Growth and Yield of Gobhi Sarson as Influenced by Irrigation and Nutrient Management Practices under Conservation Tillage

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## ABSTRACT

This study was conducted at Water Management Farm, Department of Soil Science, CSK HPKV, Palampur, India, during *rabi* 2017-18 using gobhi sarson (*Brassica napus* L.) var. GSC-7 to examine the effect of three irrigation levels (no irrigation, 50% of recommended irrigation water (25 mm depth) and 100% of recommended irrigation water (50 mm depth)) and four nutrient management practices (25 t ha<sup>-1</sup> FYM, 50% NPK+20 t ha<sup>-1</sup> FYM, 75% NPK+10 t ha<sup>-1</sup> FYM and 100%NPK) in split plot design. The irrigation at 100% recorded highest plant height (124.4 cm) and number of primary (4.58) and secondary branches (7.92) which was at par with 50% irrigation and least in rainfed plots whereas the growth parameters were recorded highest under application of 75% NPK+10 t ha<sup>-1</sup> FYM. Interaction effect was significant in case of number of primary branches per plant. The yield attributes such as number of siliquae per plant and seeds per siliqua were recorded significantly highest under 100% irrigation and application of 75% NPK+10 t ha<sup>-1</sup> FYM and least under rainfed and sole NPK plots. The 1000 seed weight (g) was non-significant under all treatments. Irrigation applied at 50 mm significantly improved seed (13.83 q ha<sup>-1</sup>) and stover (44.92 q ha<sup>-1</sup>) yield which was at par with 25 mm irrigation application. Whereas, significantly highest seed (13.50 q ha<sup>-1</sup>) and stover (43.18 q ha<sup>-1</sup>) yield was obtained under 75% NPK+10 t ha<sup>-1</sup> FYM. However, yields obtained under the sole organic and inorganic application were at par with each other.

## HIGHLIGHTS

- Application of 50 mm irrigation at critical irrigation stages along with 75% NPK+10 t ha<sup>-1</sup> was found to be more effective in improving growth, yield attributes and yield of gobhi sarson.

**Keywords:** Canola, irrigation levels, integrated nutrient management, yield, gobhi sarson

Oilseed crops stands fourth in the commodity group in the world (FAO, 2020) and second in India. Rapeseed and mustard oil stands third in the world followed by palm and soybean oil. Canada is the leader in rapeseed oil production followed by Germany and China (FAO, 2020). A massive gap between production and demand of vegetable oil exists in the country (Anonymous, 2018). Therefore, India is the major importer of vegetable oil in the world (Anonymous, 2019). To overcome this Indian agriculture is looking for emerging varieties with sustainable and low budget production system.

Gobhi sarson (*Brassica napus* L.) also known as Canola or Canadian oil crop has gained importance due to its photo and thermo sensitivity in colder regions of the country like Punjab and Himachal Pradesh replacing Indian mustard. An amphiploid between *Brassica campestris* and *Brassica oleracea*

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is a high yielding with high oil content (41-45%) variety with appreciable content of oleic, linoleic and linolenic acid. At present the productivity is marginal (12-14 q ha<sup>-1</sup>) in colder regions of Himachal Pradesh as compared to Punjab (16-18 q ha<sup>-1</sup>). The major constraint of inconsistent productivity in hilly regions is undulated small land holding. Thus, farmers' adopt low input management practices such as organic farming under rainfed conditions. About 80% of area in the state comes under rainfed conditions with inconsistent soil fertility status yielding lower productivity of agricultural crops. To overcome this sustainable interventions such as integrated nutrient management, rain water harvesting and moisture conservation should be promoted to enhance soil nutrient status. Thus, conservation tillage is a viable practice. Along with this rainwater storage and its shallow application through gravity fed irrigation system at critical irrigation stages of crop can insure irrigation under erratic rainfall conditions. Integrated nutrient management further sustain soil fertility with reducing fertilizer input by supplementing it with farm grown farm yard manure (FYM). In literature very little to no data available on sustainable nutrient and irrigation system to adopt for gobhi sarson in hilly cold climate. Coupling the two techniques of shallow irrigation and integrated nutrient management with sole organic and nutrient application along with rainfed and recommended irrigation depth this study was formulated in winter season. The objective of this study was to evaluate influence of irrigation and nutrient management on productivity and soil nutrient status of gobhi sarson (*Brassica napus* L.) under conservation tillage.

## MATERIALS AND METHODS

The experiment carried out at Water management farm, CSK HPKV, Palampur, India, comes under Agro Climatic Zone II (Mid Hills Temperate Wet) during winter 2017-18. Basically, the soils of this region are medium textured (Silty clay loam) with intermediate physical and chemical properties. The pH of soil at both depths (0-15 cm and 15-30 cm) lies at slightly acidic range. The available content of macro nutrients and organic carbon content at the initiation of the experiment was medium at both depths. The experiment was laid out in split plot design with twelve treatments. The three

irrigation levels were taken in main plot i.e., I<sub>1</sub>: No irrigation, I<sub>2</sub>: 50% of recommended irrigation water (25 mm at critical irrigation stages) and I<sub>3</sub>: 100% of recommended irrigation water (50 mm at critical irrigation stages) and four nutrient management practices in sub plots i.e., NM<sub>1</sub>: 25 t ha<sup>-1</sup> FYM, NM<sub>2</sub>: 50% NPK+20 t ha<sup>-1</sup> FYM, NM<sub>3</sub>: 75% NPK+10 t ha<sup>-1</sup> FYM and NM<sub>4</sub>: 100%NPK. The GSC-7 variety of gobhi sarson was sown with 6 kg ha<sup>-1</sup> seed by just opening of furrows 30 cm apart and crop residue of soybean from previous crop was utilized to cover the remaining area of the plot following the conservation tillage. Irrigation was applied at two critical stages i.e., 40 DAS (vegetative growth stage) and 130 DAS (siliqua development stage). FYM was applied as per treatment on a fresh weight basis at the time in the furrows before sowing. The recommended dose of N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O for the crop is 120:60:40 and was given as per the treatments. Complete dose of P and K and half dose of N was applied basal as per treatment at the time of sowing. Remaining nitrogen was applied in two equal splits at 60 DAS and flowering stages. Five plants per plot were tagged and used for measurement of growth parameter and yield attributes. Grain and straw yields of gobhi sarson were recorded at harvest. The data gathered in each observation were statistically evaluated using analysis of variance technique (Gomez and Gomez 1984). The critical differences (CD) was computed to assess the significance of treatment means at 5% level of probability.

## RESULTS AND DISCUSSION

### Growth Parameter

#### Plant height

Plant height was significantly affected by different irrigation and nutrient management practices (Table 1). The data revealed that significantly higher mean plant height of 124.4 cm were recorded when irrigation was applied at 50 mm (I<sub>3</sub>) at 40 and 130 DAS which was statistically at par with irrigation application at 25 mm (I<sub>2</sub>) depth. The lowermost plant height (85.3 cm) recorded were no irrigation (I<sub>1</sub>) was applied. Among nutrient management practices, INM gave significantly tallest plants (121.4 cm) under 75% NPK+10 t ha<sup>-1</sup> FYM followed by treatment (NM<sub>2</sub>) having plants of 110.9 cm long. The



**Table 1:** Effect of irrigation levels and nutrient management on plant height, number of primary and secondary branches per plant, number of siliquae per plant, number of seeds per siliqua and 1000-seed weight of gobhi sarson

Treatment	Growth Parameter			Yield Attributes		
	Plant height (cm)	Branches plant <sup>-1</sup> (no.)		Siliquae plant <sup>-1</sup> (no.)	Seeds siliqua <sup>-1</sup> (no.)	1000-seed weight (g)
		Primary	Secondary			
<b>Irrigation level</b>						
I <sub>1</sub>	85.3	3.24	5.00	96.2	11.21	4.37
I <sub>2</sub>	115.3	3.77	6.83	124.7	12.33	4.42
I <sub>3</sub>	124.4	4.58	7.92	139.5	14.38	4.45
<b>SEm±</b>	3.15	0.07	0.10	1.85	0.22	0.08
CD (p=0.05)	12.4	0.28	0.39	7.3	0.86	NS
<b>Nutrient Management Level</b>						
NM <sub>1</sub>	96.00	3.29	5.33	105.8	11.11	4.38
NM <sub>2</sub>	110.9	3.95	6.89	122.9	13.11	4.42
NM <sub>3</sub>	121.4	4.44	7.56	133.6	13.95	4.44
NM <sub>4</sub>	104.9	3.77	6.56	118.2	12.39	4.40
<b>SEm±</b>	3.17	0.12	0.21	5.77	0.34	0.12
CD (p=0.05)	9.4	0.35	0.62	9.9	1.01	NS
Interaction	NS	S	NS	NS	NS	NS

sole inorganic fertilizer application (NM<sub>4</sub>) recorded 104.9 cm heighted plant which was statistically at par with NM<sub>2</sub>. The treatment with 25 t ha<sup>-1</sup> FYM (NM<sub>1</sub>) recorded lowest plant height of 96.0 cm which was statistically at par with NM<sub>4</sub>.

### Number of primary and secondary branches per plant

The variable irrigation depths and nutrient management practices registered significant impact on number of primary and secondary branches plant<sup>-1</sup> at time of harvest have been presented in Table 1. Results revealed that significantly highest numbers of primary and secondary branches plant<sup>-1</sup> (4.58 and 7.92, respectively) was recorded in the treatment (I<sub>3</sub>) where 100% irrigation (50 mm) was applied at critical growth stages which was followed by 50% irrigation level (I<sub>2</sub>) with 3.77 primary and 6.83 secondary branches plant<sup>-1</sup>. The lowest primary (3.24) and secondary branches (5.00) plant<sup>-1</sup> was recorded where no extra irrigation was given at stated growth stages. Among nutrient management levels, same trend as plant height was followed where significantly highest number of primary (4.44) and secondary (7.56) branches plant<sup>-1</sup> were recorded in the treatment 75% NPK+10 t ha<sup>-1</sup> FYM (NM<sub>3</sub>) followed by treatment where 50% NPK+ 20 t

ha<sup>-1</sup> FYM (NM<sub>2</sub>) was applied though this treatment was at par with 100% NPK (NM<sub>4</sub>). Lowest number of primary (3.29) and secondary branches plant<sup>-1</sup> (5.33) were recorded in treatment where sole 25 t ha<sup>-1</sup> FYM (NM<sub>1</sub>) was applied.

The interaction effect was significant on the number of primary branches plant<sup>-1</sup> (Table 2). The number of primary branches increased with increase in irrigation frequency in all nutrient management practices. Significant increase was recorded in NM<sub>3</sub> and NM<sub>4</sub> over NM<sub>1</sub>. Treatment combination of I<sub>3</sub>NM<sub>3</sub> registered highest no. of primary branches plant<sup>-1</sup> (5.67) which was significantly superior over all other combinations of nutrient management and irrigation levels.

Application of irrigation at vegetative growth stage and siliqua development stage other than rainfall enhanced the cell turgidity, and increased meristematic tissues due to continuous moisture availability ensuring enhanced vigour and crop growth. The integrated nutrient management practice which combines both readily and slowly available nutrient sources enhanced the growth initially and over the growth period, respectively over sole organic and inorganic application. The decomposition of FYM in INM plots was augmented bring about release of growth hormones such as

**Table 2:** Interaction effect of irrigation levels and nutrient management on number of primary branches plant<sup>-1</sup>

NM	Irrigation		
	I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>
NM <sub>1</sub>	3.07	3.13	3.67
NM <sub>2</sub>	3.18	4.00	4.67
NM <sub>3</sub>	3.33	4.33	5.67
NM <sub>4</sub>	3.30	3.67	4.33
	<b>I*NM (1) <sup>†</sup></b>		<b>I*NM (2) <sup>†</sup></b>
<b>SEm±</b>	0.20	0.19	
CD (p=0.05)	0.60	0.71	

<sup>†</sup>I\*NM (1) = CD for two NM levels at the same I level; <sup>†</sup>I\*NM (2) = CD for I levels at the same or different NM level.

**Table 3:** Effect of irrigation levels and nutrient management on seed yield, stover yield and harvest index of gobhi sarson

Treatment	Yield		Harvest Index (%)
	Seed (q ha <sup>-1</sup> )	Stover (q ha <sup>-1</sup> )	
<b>Irrigation level</b>			
I <sub>1</sub>	10.27	33.69	23.47
I <sub>2</sub>	12.47	39.48	24.11
I <sub>3</sub>	13.83	44.92	23.68
<b>SEm±</b>	0.55	1.37	1.26
CD (p=0.05)	2.16	5.39	NS
<b>Nutrient Management Level</b>			
NM <sub>1</sub>	11.13	34.33	24.53
NM <sub>2</sub>	12.31	40.36	23.62
NM <sub>3</sub>	13.50	43.18	23.82
NM <sub>4</sub>	11.82	39.59	23.03
<b>SEm±</b>	0.23	1.16	0.74
CD (p=0.05)	0.68	3.46	NS
Interaction	NS	NS	NS

auxin enhanced cell elongation and cell division resulting better crop growth (Tahir *et al.* 2007; Mahajan *et al.* 2013; Tyagi and Upadhy, 2017 and Mallic *et al.* 2018).

## Yield Attributes

### Number of siliquae per plant

The influence of varying irrigation depth and nutrient management practices were significant on yield attributing characters of gobhi sarson are presented in Table 1. Application of 100% irrigation enhanced (11.86%) and (45.01%) number of siliqua per plant over 50% irrigation application and rainfed conditions, respectively. Among nutrient management levels, application of NPK with FYM yielded more number of siliquae per plant over

sole NPK and FYM application. The treatment 75% NPK+10 t ha<sup>-1</sup> FYM (NM<sub>3</sub>) yielded 9.24%, 13.03% and 26 % more number of siliquae per plant over application of 50%NPK+ 20 t ha<sup>-1</sup> FYM (NM<sub>2</sub>), 100% NPK (NM<sub>4</sub>) and FYM (NM<sub>1</sub>), respectively.

### Number of seeds per siliqua

A cursory glance at the data showed significant effect of irrigation depth on the number of seeds per siliquae. Treatment with 100% irrigation registered an increase of 16.62% and 28.28% seeds per siliquae compared to 50% irrigated and rainfed plot, respectively. Similarly, significant influence was observed with varying nutrient levels, wherein, treatment 75% NPK+10 t ha<sup>-1</sup> FYM recorded 5.87%, 11.78% and 24.66% increased seed yield per siliqua over treatments 50%NPK+ 20 t ha<sup>-1</sup> FYM



(NM<sub>2</sub>), 100% NPK (NM<sub>4</sub>) and 25 t ha<sup>-1</sup> FYM (NM<sub>1</sub>), respectively.

### 1000-seed weight

The data revealed that different irrigation levels and nutrient management practices did not significantly influence the 1000-seed weight. Among varied irrigation levels 1000-seed weight of gobhi sarson varied between 4.37 g under rainfed plots and 4.45 g with the application of 100% irrigation (I<sub>3</sub>) and the 1000-seed weight ranged between (4.38 g) under 25 t ha<sup>-1</sup> FYM (NM<sub>1</sub>) to 4.44 g in plots receiving 75% NPK+10 t ha<sup>-1</sup> FYM (NM<sub>3</sub>). The interaction effect of irrigation levels and nutrient management on yield attributing characters were insignificant.

Yield attributing characters showed significant increase with irrigation application at vegetative growth stage and siliqua development stage as compared to rainfed due to presence of adequate soil moisture at critical growth stages enhanced nutrient availability to the crop. Timely availability of moisture and nutrients might have enhanced the synthesis of photosynthates which stored in form of economic part in the crop (Hasanuzzaman and Karim 2007; Ray *et al.* 2015 and Singh *et al.* 2018). Integrated nutrient management provided higher nutrient throughout the crop growth period due to split application of nitrogen and slow decomposition of FYM provided nutrients at early as well as later stages over sole inorganic and organic application. Continuous nutrient availability in rhizosphere might be owing to better growth parameters and rapidly converting photosynthates into proteins and other components (Meena *et al.* 2013; Kumar *et al.* 2015; Sharma and Das 2017 and Singh *et al.* 2018).

### Seed and Stover Yield

A perusal of the data in table 3 revealed that seed and stover yield of gobhi sarson was significantly influenced by irrigation levels and nutrient management practices. Among irrigation levels significantly higher seed yield of 13.83 q ha<sup>-1</sup> was recorded under 50 mm irrigation (I<sub>3</sub>) which was at par with 25 mm irrigation (I<sub>2</sub>) giving seed yield of 12.47 q ha<sup>-1</sup> and rainfed plots (I<sub>1</sub>) recorded lowest seed yield of 10.27 q ha<sup>-1</sup>. The application of irrigation at critical stages enhanced the seed yield by about 34.7% at 50 mm and 21.4 % at 25 mm in

comparison to that under rainfed plots. The trend for stover yield was also quiet similar where 100% irrigation application yielded significantly highest stover yield (44.92 q ha<sup>-1</sup>) which was followed by 50% irrigation with 39.48 q ha<sup>-1</sup> and lowest (33.69 q ha<sup>-1</sup>) was recorded under rainfed plots. Crop under irrigated condition thrived due to timely presence of soil moisture at all critical stages which might have enhanced leaf water potential, stomatal conductance, light absorption, leaf area index and nutrient absorption which have positive impact on the yield attributes ultimately leading to higher yield (Panda *et al.* 2004; Mandal *et al.* 2006; Sarkar and Sarkar, 2017 and Sharma and Das 2017).

Among nutrient management levels, integrated plots yielded significantly highest seed and stover yield over sole organic and inorganic plots. Plots with 75% NPK+10 t ha<sup>-1</sup> FYM (NM<sub>3</sub>) yielded significantly highest seed yield of 13.50 q ha<sup>-1</sup> followed by the application of 50% NPK+ 20 t ha<sup>-1</sup> FYM (NM<sub>2</sub>) giving 12.31 q ha<sup>-1</sup>. Plots with 75% NPK+10 t ha<sup>-1</sup> FYM (NM<sub>3</sub>) yielded highest stover yield of 43.18 q ha<sup>-1</sup> but was at par with application of 50%NPK+ 20 t ha<sup>-1</sup> FYM (NM<sub>2</sub>). Plots under 25 t ha<sup>-1</sup> FYM (NM<sub>1</sub>) treatment gave the lowest seed and stover yield of 11.13 q ha<sup>-1</sup> and 34.33 q ha<sup>-1</sup>, respectively. Application of 100% NPK was 5.83 and 13.2 per cent superior in seed and stover yield, respectively over sole organic plots which might be due presence of readily available form of nutrients in inorganic form are present at early growth stages as compared to slower decomposition rate of FYM slowing down growth at vegetative stage. Nutrient management level NM<sub>3</sub> out yielded because of adequate availability of macro nutrients from both fertilizers and FYM throughout the growing period along with micro nutrients which might played a crucial role in biological activity by stimulating enzymatic reactions in rhizosphere indirectly enhancing nutrient availability to the crop resulting to produce more yields (Hati *et al.* 2001; Ali *et al.* 2003; Mandal *et al.* 2006 and Singh *et al.* 2018). The interaction effect between irrigation levels and nutrient management was not significant with respect to seed and stover yield of gobhi sarson. There was a non-significant impact of irrigation and nutrient management on the harvest index, and all the treatments showed an equal effect on the harvest index of the crop.



## CONCLUSION

Results of the study concluded that irrigation at 50 mm depth at critical irrigation stages (vegetative growth stage and siliqua development stage) combined with application of 75% NPK+10 t ha<sup>-1</sup> FYM (I<sub>3</sub>NM<sub>3</sub>) gave higher growth parameters viz. plant height number of primary and secondary branches per plant and yield parameters viz. no. siliquae per plant, number of seeds per siliqua and 1000-seed, seed yield and stover yield.

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# Impact of Heat Units and different Pruning Months on Growth and Flowering of *Jasminum grandiflorum*

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## ABSTRACT

An investigation was carried out at the Department of Floriculture & Landscaping, TNAU, Coimbatore to study the effect of Heat units on pruning, growth and flowering of *Jasminum grandiflorum*. The level of pruning height was 30 cm from the ground and pruning months (September-April) every month was done except in the peak season (May-August) of flowering. Pruning during the last week of October gave the highest plant height (65.18 cm), number of shoots (47.33) and maximum yield (64.24 g/plant) in *Jasminum grandiflorum* whereas, the lowest plant height (48.9 cm), number of shoots (27.00) was observed in November and yield (24.59 g/plant) December pruning. The meteorological parameters viz., maximum temperature (2017-31.4 and 2018-30.4°C), minimum temperature (2017-22.9 and 2018-22.4°C), relative humidity (2017- 88 and 2018-87%), sunshine hours (2017-6.2 and 2018-8.2) day length (2017-12.08 and 2018-12.09), GDD (2017-0.30 and 2018-0.29), PTU (2017-3.62 and 2018-3.51), HTU (2017-1.86 and 2018-2.38) and HUE (2017-456.30 and 2018-362.70 kg ha<sup>-1</sup> day °C) were recorded under field conditions.

## HIGHLIGHTS

- Recording daily temperatures of the plants with the help of infrared thermometer, continuous pruning of plants for eight months except in peak season of flowering calculation of PTH, HTU, GDD and HUE of the crop based on changing climatic conditions. Observation of growth and yield parameters of *Jasminum grandiflorum* plants.

**Keywords:** *Jasminum grandiflorum*, pruning height, pruning months, growth, yield, Heat units

Jasmine is one of the important fragrant flowers used even from very ancient days in India. It is highly esteemed for its attractive, white-coloured and fragrant flower and has a pride of place in the heart of every south Indian woman. In the Fragrance industry, jasmine has unique importance and popularity due to its unique sweet fragrance like that of rose, and vetiver and represents a type that cannot be exactly imitated at present by a mixture of any known synthetic aroma chemicals or natural isolates. The extracts of jasmine are used for flavouring or preparation of 'Jasmine scented Tea' in China and 'Jasmine rice' in Bangkok, Thailand. The antioxidant properties can induce weight loss and reduce serum and hepatic lipid levels through

the increase of leptin levels which addresses the burning problems of fattiness and obesity (Li Zhen *et al.* 2011). Jasmine will emerge as an important "Industrial flower crop". The essential oil is being used in cosmetics, perfumery and as a source of aroma chemicals and food flavouring industries. It is grown in the 'Grasse region' of Southern France, Syria, Algeria, Sicily, Calabria and Morocco apart from India. India exports fresh jasmine flowers to neighbouring countries like Sri Lanka, Singapore,

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Malaysia and the Gulf countries. Jasmine flower crop is grown on a commercial scale throughout India, but extensively in Tamil Nadu (12590 ha area and 1,30,070 MT production, 2015-16), Karnataka (5760 ha area 3,69,200 MT), Andhra Pradesh (2710 ha area and 1,51,300 MT).

Being an industrial flower crop large quantities of flowers need to be produced continuously for a longer period of the year to meet the break-even production of the essential oil industry. Hence, the flower production programme needs to be scheduled to further escalate the area under the jasmine flower crop. The large quantitative production requirement can be achieved by area expansion and increasing productivity. This study has been undertaken to study the Influence of different pruning months and pruning height on the growth and flowering of *Jasminum grandiflorum*.

## MATERIALS AND METHODS

A field experiment was conducted in the farmers' field at Mathampalaiyam village, Coimbatore, from 2017 to 2019. The experiment was laid out in Randomized Block Design (RBD) with 8 treatments and 3 replications. Five-year-old jasmine plants were selected for investigation. Treatment includes pruning of jasmine plants at different months *viz.*, during the last week of September, October, November, December, January, February, March and April. Pruning was done by cutting back all past-season shoots at 30cm from the ground level at the farmer's request for their benefit. The main objective of this study is "Influence of different pruning months on growth and flowering of *Jasminum grandiflorum*". The pruned plants were observed for vegetative growth like plant height no of shoots and flower quality parameters *viz.*, a total length of flower bud and length of a flower bud (without corolla tube). Five randomly selected plants were tagged per replication in each treatment and observations were recorded. The statistical analysis was done following the method of Panse and Sukhatme (1978).

### Metrological parameters (Monthly)

#### Effect of climatic factors on growth and flowering of *Jasminum grandiflorum*

Heat unit system is a scheme for studying plant

growth and temperature relationship by the accumulation of daily mean temperature above a certain threshold temperature during the growing season.

### Growing Degree Day (GDD)

A degree day or a heat unit is the departure from the mean daily temperature, above the threshold temperature of the crop. The GDD are calculated by the quotation.

$$GDD = \sum \frac{(T_{max} + T_{min})}{2 - T_t}$$

It means, sum of different between mean temperature and base temperature or threshold temperature. The threshold temperature is the temperature below which no growth takes place. This varies with different crops, generally higher values for tropical crops and lower values for temperature crops.

### Photo Thermal Unit (PTU)

The GDD concept applies day length factor to improve the flowering. This is calculated by multiplying GDD with day length or maximum possible bright sunshine hours (N) as suggested by (Nuttonson, 1948).

$$PTU = \sum GDD \times \text{Maximum possible bright sunshine hours}$$

### Heat Use Efficiency (HUE)

It has been defined as yield per day °C on growing day concept or per unit of day °C hours on helio-thermal units indicating the efficiency with the available heat utilized for flower yields (unit: kg/ha day °C).

$$HUE = \frac{\text{Yield}}{GDD}$$

## RESULTS AND DISCUSSION

### Influence of pruning on plant vegetative growth characters of *Jasminum grandiflorum*

The results from Table 1 revealed the effect of the month of pruning and pruning height on growth parameters *viz.*, plant height, plant spread, and the

number of shoots per plant during the offseason (December to February) is found significant. The plant pruned at 30 cm from ground level in October recorded the highest plant height (65.18 cm) and a maximum number of shoots per plant (47.33) when compared with plants pruned in November. This may be because the maximum temperature during October month is 30.4° C and the day length is about 11.5 hours. According to Leonhardt and Teves, (2002), Rai (1984) and Pal and Krishnamurthi, (1967) jasmine needs a maximum temperature of 30° C and long days and cumulative heat are favourable for growth and flower induction. The lowest height (48.9 cm) and less number of shoots per plant (27.00) were observed in plants pruned during the last week of November T<sub>2</sub> because the weather was not in the favour of jasmine which needs a maximum temperature of 30° C and long days and cumulative heat are favourable for growth and flower induction.

#### Micro climate of the plant Leaf temperature (°C)

The leaf temperature (°C) parameters during plant growth were influenced by different months of pruning as furnished in Table 1. Regarding different months of pruning showed significant influence on the leaf temperature (°C). Among the different months of pruning, the threshold leaf temperature (°C) for jasmine growth and profuse flowering was observed in T<sub>8</sub> (pruning during last week of September) recorded 27.53°C followed by T<sub>1</sub> (pruning during last week of October) which recorded 27.93°C. The treatment T<sub>3</sub> (pruning during last week of December) recorded the lowest leaf temperature 26.13°C.

The effect of early pruning during October last week to make the plants being able to receive longer photoperiodic stimulus than the late pruned ones where the day lengths were shorter and there was a drastic reduction in the mean temperatures which resulted in stunted growth is in agreement with the findings of Kalaimani, 2017; Sujatha *et al.* (2009), Jennoah, 2012 in *Jasminum sambac*. Whereas T<sub>2</sub> (pruning during the last week of November) produced the shortest plants (48.9) at the flowering stage. This might be due to the effect of weather conditions with low heat units which leads to low photosynthesis and restrict the cell enlargement.

This finding aligns with the result of Kumaresan, 2016 and Chaitanya 2013 in *Jasminum sambac*.

**Table 1: Influence of different months of pruning on plant height and number of shoots per plant (30 days after pruning)**

Treatments (Pruning months)	Plant height (cm)	Number of shoots per plant
T <sub>1</sub> (October)	65.18	47.33
T <sub>2</sub> (November)	48.9	27.00
T <sub>3</sub> (December)	51	31.67
T <sub>4</sub> (January)	53.41	44.67
T <sub>5</sub> (February)	54.31	42.00
T <sub>6</sub> (July)	51.96	33.67
T <sub>7</sub> (August)	50.02	32.00
T <sub>8</sub> (September)	57.16	46.00
<b>Mean</b>	<b>53.99</b>	<b>38.04</b>
<b>CD (5%)</b>	<b>1.43</b>	<b>3.23</b>
<b>Sed</b>	<b>0.71</b>	<b>1.61</b>

Pruning treatments significantly increased the plant spread which might be due to the suppression of apical dominance that produced a greater number of main and lateral branches, resulting in increased plant spread in both directions as observed by Kumaresan, 2016 in *Jasminum sambac*.

#### Influence of pruning on flowering and yield parameters of *Jasminum grandiflorum*

The effect of pruning on flowering parameters (days taken for the first harvest of flower bud, number of flowers per cymes, the weight of 100 flower buds, flower yield per plant) was found to be significant and presented in Table 2.

The mean earliest days taken for the first harvest of flower buds (45.00 days) were observed in T<sub>1</sub> (pruning during the last week of October). Among the different months of pruning the maximum bud length (3.10 cm), a maximum weight of 100 buds (20.10 g) and the highest yield (64.24 g/plant) were observed in T<sub>1</sub> (when plants were pruned in the last week of October) followed by plants pruned during last week of September T<sub>8</sub> and the lowest was observed in plant pruned during last week of November T<sub>2</sub>. Because in October the plants are exposed to a temperature of 30.4° C and long days which leads to profuse flowering Pal and Krishnamurthi (1967).



**Table 2:** Influence of different levels of pruning and months of pruning on floral characters of *Jasminum grandiflorum*

Treatments (Pruning months)	Total bud length (cm)	Weight of 100 flower buds (g)	Yield g/ plant
T <sub>1</sub> (October)	3.10	20.10	64.24
T <sub>2</sub> (November)	2.50	13.14	26.73
T <sub>3</sub> (December)	2.40	10.49	24.59
T <sub>4</sub> (January)	2.80	17.64	50.64
T <sub>5</sub> (February)	2.87	19.15	45.28
T <sub>6</sub> (July)	2.70	16.83	32.26
T <sub>7</sub> (August)	2.63	16.09	29.07
T <sub>8</sub> (September)	2.90	18.61	62.93
<b>Mean</b>	<b>2.74</b>	<b>16.51</b>	<b>41.97</b>
<b>CD (5%)</b>	<b>0.50</b>	<b>0.97</b>	<b>1.19</b>
<b>Sed</b>	<b>0.25</b>	<b>0.48</b>	<b>0.59</b>

A maximum number of branches might have ultimately resulted in an increased yield of flower buds. Flower yield is dependent on the number of flowering branches. Production of more amount of foliage in October and September pruned plants might have resulted in increased photosynthesis and ultimately large reserve food source leading to the production of a greater number of flowers as reported by Kumaresan, (2016), Chaitanya, (2013) Sujatha *et al.* (2009) and Jennoah, (2012) in *Jasminum sambac*.

Metrological parameters 4.3.5.1. Growing Degree Day (GDD) The growing degree day for *J.*

*grandiflorum* was calculated and given in the below Table. Among different months of pruning, the plants T<sub>8</sub> pruned during last week of September gave maximum yield with a GDD of 2017- 0.30 and in 2018- 0.29 has recorded and followed by T<sub>1</sub> pruned during last week of October with a GDD of 2017- 0.31 and 2018- 0.39. And lowest yield was observed in T<sub>3</sub> (pruned during last week of December) with a GDD of 2017-0.31 and 2018-0.33. 4.3.5.2. Photo Thermal Unit (PTU) The photo thermal unit (PTU) for *J. grandiflorum* was calculated and given in table. Regarding various months of pruning, the plants, T<sub>8</sub> pruned during last week of September gave maximum yield with a PTU of 2017- 3.62 and in 2018- 3.51 has recorded and followed by T<sub>1</sub> pruned during last week of October with a PTU of 2017- 3.57 and 2018- 4.49. And lowest yield was observed in T<sub>3</sub> (pruned during last week of December) with a PTU of 2017-3.49 and 2018-3.72. 4.3.5.3. Helio Thermal Unit (HTU) The Helio Thermal Unit (HTU) for *J. grandiflorum* was calculated and given in below Table. Of various months of pruning, the plants, T<sub>8</sub> pruned during last week of September gave maximum yield with a HTU of 2017- 1.86 and in 2018- 2.38 has recorded and followed by T<sub>1</sub> pruned during last week of October with a HTU of 2017- 1.77 and 2018- 2.69. And lowest yield was observed in T<sub>3</sub> (pruned during last week of December) with a HTU of 2017-1.83 and 2018-1.62.

**Table 3:** Effect of GDD, PTU and HTU of the *Jasminum grandiflorum*

Months	GDD			PTU			HTU		
	2017	2018	2019	2017	2018	2019	2017	2018	2019
January	0.37	0.41	0.43	4.18	4.64	4.88	2.70	2.95	4.17
February	0.46	0.46	0.42	5.26	5.26	4.82	3.36	4.09	3.49
March	0.41	0.43	0.44	4.86	5.10	5.25	3.77	3.53	4.22
April	0.43	0.38		5.25	4.64		3.83	3.27	
May	0.39	0.33		4.82	4.08		3.00	3.17	
June	0.33	0.24		4.10	2.98		1.75	1.01	
July	0.32	0.25		3.96	3.10		1.92	1.40	
August	0.25	0.25		3.07	3.07		1.10	1.18	
September	0.30	0.29		3.62	3.51		1.86	2.38	
October	0.31	0.39		3.57	4.49		1.77	2.69	
November	0.23	0.22		2.84	3.06		1.35	1.73	
December	0.31	0.33		3.49	3.72		1.83	1.62	
Mean	0.35	0.34	0.43	4.09	3.97	4.98	2.35	2.42	3.96

GDD – Growing degree days PTU – Photo thermal units HTU – Helio thermal units





Fig. 1: *Jasminum grandiflorum* at 30 cm above ground level

### Heat Use Efficiency (HUE)

The Heat Use Efficiency (HUE) for *J. grandiflorum* was calculated and presented in table. Of various months of pruning, the plants, T<sub>8</sub> plants pruned during last week of September registered maximum

yield with a HUE of 466.18 and 482.25 in 2017 and 2018 respectively has recorded and followed by T<sub>8</sub> pruned during last week of October with a HUE of 2017-456.30 and 2018-362.70. And lowest yield was observed in T<sub>3</sub> (pruned during last week of December) with a HUE of 2017-256.43 and 2018-240.89.

Table 4: Heat use efficiency (kg ha<sup>-1</sup> day °C) of *J. grandiflorum*

Treatments	2017	2018
T <sub>1</sub>	456.30	362.70
T <sub>2</sub>	394.60	412.53
T <sub>3</sub>	256.43	240.89
T <sub>4</sub>	386.54	348.83
T <sub>5</sub>	305.11	305.11
T <sub>6</sub>	261.52	249.35
T <sub>7</sub>	230.29	260.59
T <sub>8</sub>	466.18	482.25
Mean	344.62	332.78

T<sub>1</sub>: October T<sub>2</sub>: November T<sub>3</sub>: December T<sub>4</sub>: January; T<sub>5</sub>: February  
T<sub>6</sub>: March T<sub>7</sub>: April T<sub>8</sub>: September.



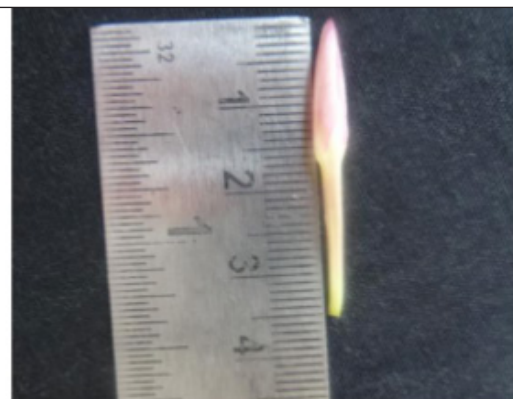
Plants pruned during September



Plants pruned during December



*Jasminum grandiflorum* September pruning



*Jasminum grandiflorum* December pruning

Fig. 2: Effect of different months of pruning in *Jasminum grandiflorum*



## CONCLUSION

The present study led to the conclusion that pruning the last week of October at a height of 30 cm above ground level gave ample results. This is due to changes in weather conditions like changes in temperature and day length prevailed during October month. Hence, according to changes in weather the pruning month should be changed to get an ample production and yield in *Jasminum grandiflorum*.

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# Assessing Socio-economic Vulnerability for Development: Evidence from Ahmednagar, Maharashtra

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## ABSTRACT

Vulnerability assessment is vital for developing appropriate adaptation measures to improve community resilience. This research aims to assess the socio-economic vulnerability of the Ahmednagar district in Maharashtra state using a novel Socio-Economic Vulnerability Index (SEVI). Ahmednagar is one of the most populous and progressive districts with a high Human Development Index (HDI) value (0.72). This study covered all the 14 tehsils of the Ahmednagar district and used secondary data sources for assessing sensitivity and adaptive capacity. Ahmednagar district had low social sensitivity (0.28) with moderate social adaptive capacity (0.48) whereas high economic sensitivity (0.63) with low economic adaptive capacity (0.38). Overall, the SEVI score of Ahmednagar district was low (0.30). However, tehsil level analysis showed high vulnerability scores for 5 tehsils namely Nevasa, Shirampur, Shevgaon, Rahuri and Jamkhed, which need special attention on social sensitivity aspects. The one-way ANOVA test also revealed statistically significant differences among tehsils in terms of each of the SEVI indicators, suggesting location-specific interventions to reduce sensitivity and increase adaptive capacity. The study identified key drivers that pushed sensitivity up (higher proportion of small and marginal farmers; longer distance to town and hospital) and lowered adaptive capacity (poor housing condition and amenities; poor transport and communication; less livestock population) in identified highly vulnerable tehsils for concerted efforts.

## HIGHLIGHTS

- Ahmednagar district was categorised as *less* socio-economically vulnerable with a SEVI score of 0.30.
- Higher degree of variability existed among 14 tehsils, with 5 of them found to be *highly* vulnerable.
- SEVI framework is a useful tool for developing grassroots and location-specific interventions (tehsils / villages) for reducing vulnerability and improving human development.

**Keywords:** Socio-economic Vulnerability, Adaptive Capacity, Sensitivity, SEVI, Ahmednagar

India was placed at 131<sup>st</sup> position out of 189 countries in the Human Development Report 2020, and placed 66<sup>th</sup> out of 109 nations in the Multidimensional Poverty Index 2021 (UNDP, 2020). Though 273 million Indians were lifted out of poverty in just ten years (2005-2015), income disparity has increased in the last 20 years (OECD, 2022). The top 10% of people earn 12 times as much as the bottom 10%. This disparity persists across the country and between states, as well as between districts. Even in areas with relatively high incomes and adaptive capacities, specific groups of individuals, such as women, children, and the

elderly, can be particularly vulnerable. In India, formal and informal systems and institutions shape the capabilities differently of men and women and of people from various communities. The stratified caste system, for example, has an impact on an individual's right to access resources; persons from lower castes are "the poorest economic component

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of rural life,” with “restricted access to education and financial institutions, as well as little effective voice” (Jones and Boyd 2011; Nielsen and Reenberg 2010; Simmons and Supri 1997; Sugden *et al.* 2014).

Vulnerability is defined as “the degree to which a system is sensitive to, or unable to cope with, detrimental effects of climate change, including climate variability and extremes” (IPCC, 2014). Vulnerability is determined by the type, magnitude, and rate of climate change to which a system is exposed, as well as its sensitivity and adaptive capacity (IPCC, 2001). Vulnerability (to climate change) is defined by the IPCC as a function of a system’s exposure and sensitivity to climatic stimuli, as well as its ability to adapt to their negative consequences (IPCC 2007).

$$V(\text{Vulnerability}) = f(\text{potential impact} - \text{adaptive capacity})$$

A higher adaptive capacity is associated with a lower vulnerability, while a higher sensitivity is associated with a higher vulnerability (IPCC).

“Vulnerability” has become a buzz word in recent years. It’s linked to natural risks, social hazards, or a combination of the two: sea-level rise (SLR), coastal inundation, floods, and storm surges are examples of natural hazards, while poverty, population, and other socioeconomic factors are examples of social hazards (Donner and Rodríguez, 2011). Vulnerability studies are crucial at a micro level (tehsil) because they help to understand the vulnerability of the people who live there, as well as provide avenues for their vulnerability preparedness and development. According to Krishnan *et al.* (2019), the socio-economic vulnerability of persons was measured as a function of their sensitivity and adaptive capacity. The sensitivity and adaptive capacity indicators were further divided into four categories based on the dimensions they were supposed to measure: social sensitivity, economic sensitivity, social-adaptive capacity, and economic-adaptive capacity.

### Vulnerability and its assessment

The vulnerability is determined by a combination of environmental, socio-political, and geographical factors. Non-climate issues such as poverty, inequality, food insecurity, violence, disease, and

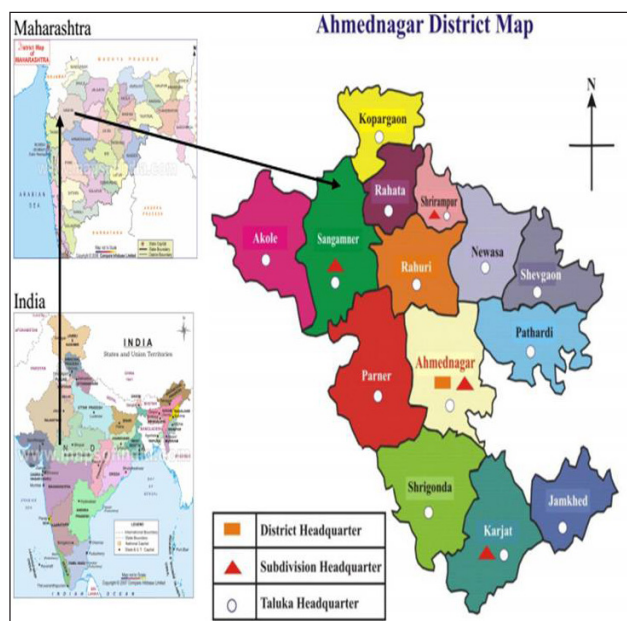
globalization can all enhance vulnerability by influencing systems’, communities’, and individuals’ exposure, sensitivity, and adaptive capacity (Adger *et al.* 2001). Adaptive capability is the prerequisite for change adaptation. Adaptive capacity is increased by practical strategies of dealing with climatic changes and uncertainties, such as variability and extremes (Smit and Pilifosova 2003). Turner *et al.* (2003) offered a vulnerability framework in which they emphasized the complexity of vulnerability assessment and advised the inclusion of factors such as identifying the complexity, nesting of scales, and potential new hazards. Vulnerability is defined as a person’s or group’s characteristics and situation that influence their ability to predict, cope with, resist, and recover from the impact of natural disasters (Blaikie *et al.* 2004). Historically, the poor and marginalized have been the most vulnerable to natural disasters, and this vulnerability will be exacerbated by climate change (Parry *et al.* 2007). The vulnerability is determined not only by the distribution of climate impacts (exposure), but also by the sensitivity and adaptive capacity of the individuals. As a result, vulnerability is socially differentiated: almost all weather-related hazards associated with climate variability, as well as human sources of vulnerability, affect different groups in society differentially (Daw *et al.* 2009; Rao *et al.* 2013). The vulnerability assessments can be utilized for a variety of goals, including strengthening adaptation planning, raising awareness of risks and opportunities, and advancing scientific research (Patt *et al.* 2010). According to Guiteras *et al.* (2009), climate change will cut main agricultural yields by 4.5 to 9% between 2010 and 2039. The long-term impact (2070-2099) is expected to be severe, with yields falling by 25% or more. Climate change would diminish essential agricultural production by 4.5 to 9% between 2010 and 2039. Long-term effects (2070-2099) are substantial, with yields estimated to drop by 25% or more.

Vulnerability evaluations are subjective and differ depending on geography and hazard. However, the inclusion of contributing elements in the vulnerability-assessment framework is dependent on data availability and the study environment (Sehgal *et al.* 2017). Vulnerability assessments are frequently based on both current and future climate change scenarios. Very few studies have

sought to understand socioeconomic vulnerability at the district or sub-district level in order to tailor development to minimize vulnerability and/or build resilience (Seenivasan *et al.* 2022). The study concentrated on the socio-economic dimension of vulnerability because these are the primary areas of intervention for strengthening adaptive capacity. The objective of the study was to examine the socio-economic vulnerability of Ahmednagar district at tehsils level, as well as to offer evidence-based strategies to reduce vulnerability.

## MATERIALS AND METHODS

Maharashtra's Ahmednagar is one of the state's most populous districts (41.24 lakh; 232 people/km<sup>2</sup>). Located between 18°2' and 19°9' north latitude and 73°9' and 75°5' east longitude in Nashik division, it is spread over a geographical area of 17.02 lakh hectares (5.66% of the state's area). The district is divided into 14 tehsils (Unit of study) (*Census tables | Government of India, 2011*). The tehsil map of Ahmednagar is shown in Fig. 1. With a relatively high HDI value (0.72), Ahmednagar is ranked 17 out of 36 Maharashtra districts.



Source: Department of Agriculture, Government of Maharashtra

Fig. 1: Map of Ahmednagar

### Socio-economic vulnerability index (SEVI) - Framework

To measure socioeconomic sensitivity and adaptive capacity, the Socio-economic Vulnerability Index

framework developed by Krishnan *et al.* (2019) was used. The methodology has been used for assessing the coastal community's vulnerability to climate change, but it is scalable across geographies and hazards within the Indian context. The SEVI framework (Fig. 2) is also adaptable, allowing for the inclusion of additional indications particular to a given scenario. The framework is being utilized to analyze the socioeconomic vulnerability of Ahmednagar district, which will aid in selecting the most vulnerable areas for priority attention. Sensitivity (S), adaptive capacity (AC), and exposure are important factors in determining the vulnerability of households and communities to the effects of climate variability and change. However, vulnerability obtained from S and AC indicators reveals people's vulnerability to their social and economic conditions. There are 22 indicators in the SEVI framework. Table 1 provides a list of indicators, their nature of relationship with sub-dimensions and different data sources.

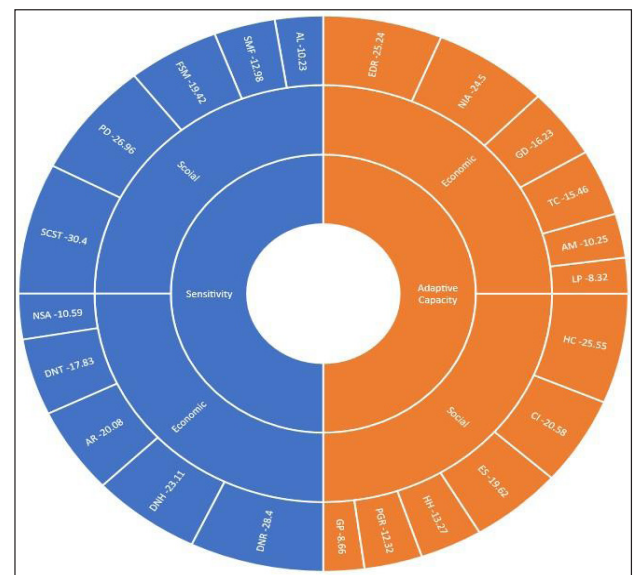


Fig. 2: Socio-economic vulnerability Index (SEVI) Framework with indicators and their weightage

SMF- Small and Marginal Farmers, AL- Agricultural Laborers, FSM-Food Sufficiency/Malnutrition, PD-Population Density, SCST- Schedule Castes/Schedule Tribes Population, NSA- Net Sown Area, AR- Annual Rainfall, DNR- Dependence on Natural Resources, DNT- Distance to Nearest Town, DNH- Distance to Nearest Hospital, TC- Transport and Communication, EDR- Economic Dependency Ratio, AM- Access to Market, NIA- Net Irrigated Area, GD- Groundwater Development, LP- Livestock Population, ES- Education Status, HH- Household Amenities, HC- Housing Condition, CI- Community Infrastructure, PGR- Population Growth Rate, GR- Gender Ratio (Krishnan *et al.* 2019).

**Table 1:** SEVI indicators, their nature of relationship and data sources

Sub- Dimension	Social	Relationship	Data sources	Economic	Relationship	Data sources
Sensitivity (SI)	SMF	Direct	AC	NSA	Direct	CI
	AL	Direct	AC	AR	Inverse	IMD
	FSM	Direct	NFHS	DNR	Inverse	CI
	PD	Direct	CI	DNT	Direct	CI
	SCST	Inverse	CI	DNH	Direct	CI
Adaptive capacity (ACI)	ES	Direct	CI	AM	Direct	CI
	HH	Direct	CI	NIA	Direct	CI
	HC	Direct	CI	GD	Direct	CGB
	CI	Direct	CI	LP	Direct	LC
	PGR	Inverse	CI	EDR	Inverse	CI
	GP	Direct	CI	TC	Direct	CI

**Legend:** CI- Census of India (2011&2001), AC – Agricultural census (2010-2011), NFHS- National family health survey (2015-2016), IMD- Indian Meteorological department (1951-2012), CGB- Central groundwater board (2011-2012), LC- Livestock census (2012).

### Normalization of indicators

To address the issue of incommensurability of the units used to quantify the various indicators during their integration into the aggregate vulnerability index, the data were normalized to a common (analogous) unit-less scale (0 to 1). Normalization was performed for each indication based on its relationship to the broad categories as specified in the formulae;

$$Z_i = \frac{X_i - X_{\min}}{X_{\max} - X_{\min}} \quad \dots(1)$$

$$Z_i = \frac{X_{\max} - X_i}{X_{\max} - X_{\min}} \quad \dots(2)$$

For directly and inversely related indicators, Eqs. 1 and 2 were used, respectively.  $Z_i$  is the normalized value of the  $i^{\text{th}}$  tehsil in relation to indicator  $X$ , and  $X_i$  is the indicator's value in original units for the  $i^{\text{th}}$  tehsil. The universal minimum and maximum values were designated by  $X_{\min}$  and  $X_{\max}$ . SI, ACI, and SEVI were rescaled on a relative basis to enable grassroots level (tehsil) planning and interventions, with an observed minimum and maximum retained as  $X_{\min}$  and  $X_{\max}$  values. The following formula was used to measure the socio-economic vulnerability index (SEVI);

$$SEVI = \frac{\text{Sensitivity Index}}{1 + \text{Adaptive Capacity Index}}$$

### Rescaled socio-economic vulnerability index (SEVI)

In order to discern greater variability to enable grassroots level (tehsils) planning and interventions, SI, ACI, and SEVI were rescaled on a relative basis, keeping observed minimum and maximum as  $X_{\min}$  and  $X_{\max}$  values. For all indices viz., SI, ACI, and SEVI, a four-point ordered scale was used to rank from very low (0 to 0.25) to very high (0.75-1.0) according to their functional relationship with vulnerability. SEVI for all the tehsils were constructed from the rescaled sensitivity (SI-R) and adaptive capacity (ACI-R) indices.

## RESULTS AND DISCUSSION

### Social sensitivity

Overall, Ahmednagar district has less social sensitivity (0.28), indicating favorable demographic characteristics. Due to a smaller proportion of SC/ST population (24%) and agricultural laborers (25%) pulled the S-SI value down. For all 14 tehsils, social sensitivity score was low, ranging from 0.22 (Parner - extremely low) to 0.41 (Akola - low). The proportion of SC/ST population in Akola tehsil was quite high (66.7%), which pushed social sensitivity by driving index value up. Small and marginal farmers own larger net sown areas in Nevasa (64%) than in Akola (38%). Rahta had the highest population density (450 people/Km<sup>2</sup>) while Parner

had the least population density (164 people/Km<sup>2</sup>) among the all tehsils.

### Economic sensitivity

The Ahmednagar economic sensitivity was found to be high (0.63). The longer distances to the nearest towns and the hospitals, as well as the limited dependence on natural resources, enhanced E-SI score for Ahmednagar, pushing the index values higher. Lower dependence on natural resources (5%) in Ahmednagar meant lesser extent of forests, pastures, and other buffering resources, signaling higher sensitivity to cope up in adverse scenarios and scope for alternative livelihood options. Ahmednagar has a significantly higher economic sensitivity due to its high NSA (74%) while most of the rural places in Ahmednagar are located far from the urban places and hospitals (>10 km). The economic sensitivity index (ESI) was found to be high in Shevgaon and low in Rahta and Sangamner tehsils, ranging from 0.68 to 0.57. Net sown area was highest in Nevasa (89%) and least in Akola and Sangamner (61%).

### Social adaptive capacity

The social adaptive capacity indicates moderate vulnerability levels (0.48). In Ahmednagar, high education status (70%), low decadal population growth rate (24%), and improved community infrastructure (78%) led to relatively higher social adaptive capacity index value. The percentage of households with access to electricity is 64% with more than 90% of households having own houses, although sanitation (15%) and safe drinking water (24%) availability was found to be very

limited. Overall, social adaptive capacity (S-ACI) of Ahmednagar tehsils ranged from poor to high across tehsils, with values ranging from 0.42 in Jamkhed to 0.53 in Nagar. The population growth rate in Nagar tehsils was reasonably high (71%) but very low in Akola (10%). Rahta had the highest literacy rate among all tehsils (81%).

### Economic adaptive capacity

The economic adaptive capacity index was determined to be low (0.38), because of weak market access, a much lower livestock population, and a modest economic dependency ratio. The average economic dependency ratio and abundant groundwater availability improved the economic adaptive capacity score. The economic adaptive capacity index was modest for all tehsils, ranging from 0.29 in Nevasa to 0.45 in Akola. In Akola tehsil, transportation and communication were extremely limited as barely 9% of settlements had adequate transportation and connectivity. Rahta was a single tehsil with superior market access (61%). Kopergaon had a higher net irrigated area (61%) than Shrigonda (8%). Almost all tehsils had greater access to groundwater (Table 2).

### Socio-economic vulnerability status of Ahmednagar

Based on the SEVI framework, Ahmednagar was found to be less vulnerable, with a mean index value of 0.30. It was the cumulative result of low social sensitivity (0.28), moderate social adaptive capacity (0.48), high economic sensitivity (0.63) and low economic adaptive capacity (0.38). Overall, sensitivity (0.44) and adaptive capacity (0.45) was similar for Ahmednagar district (table. 3). Akola

**Table 2:** Tehsil-wise indices of Ahmednagar

Tehsils	Akola	Sangamner	Kopergaon	Rahta	Shrirampur	Nevasa	Shevgaon	Pathardi	Nagar	Rahuri	Parner	Shrigonda	Karjat	Jamkhed	Ahmednagar*
S-SI	<b>0.41</b>	0.28	0.31	0.3	0.32	0.3	0.27	0.23	0.25	0.31	0.22	0.26	0.24	0.23	0.28
E-SI	0.62	0.57	0.63	0.57	0.63	0.66	<b>0.68</b>	0.66	0.62	0.61	0.63	0.63	0.63	0.67	0.63
S-ACI	0.47	0.51	0.51	<b>0.54</b>	0.51	0.49	0.45	0.43	0.53	0.51	0.51	0.49	0.45	0.42	0.48
E-ACI	<b>0.45</b>	0.38	0.44	0.37	0.31	0.29	0.35	0.40	0.33	0.32	0.43	0.38	0.38	0.41	0.38

Note; \*- District.

and Nevasa tehsils of Ahmednagar exhibited the highest SEVI score (0.33) among 14 tehsils, even though they fell in the *low* vulnerability category. Sangamner, Rahta, Parner tehsils had the least SEVI scores (0.28) (Fig. 3).

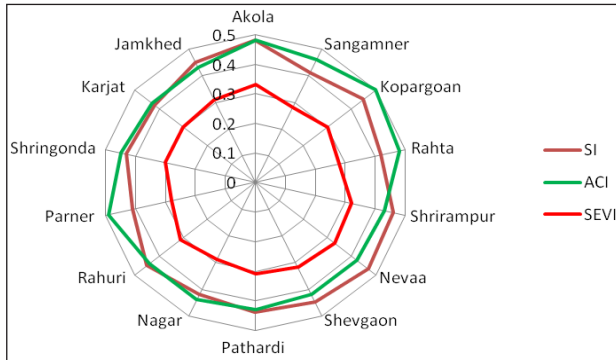


Fig. 3: SI, ACI, and SEVI values of Ahmednagar tehsils

Table 3: Overall SEVI score of Ahmednagar

	Sensitivity		Adaptive capacity		SI	ACI	SEVI
	Social	Economic	Social	Economic			
Ahmednagar	0.28	0.63	0.48	0.38	0.44	0.45	0.30

Note: SI- Sensitivity Index; ACI- Adaptive Capacity Index; SEVI- Socio-economic Vulnerability Index.

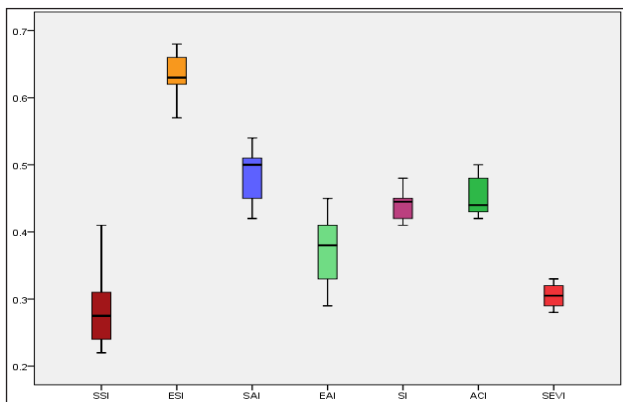


Fig. 4: Box plot of SEVI indices

Box plot was plotted for all the sub-indices used for social and economic dimensions of both sensitivity and adaptive capacity components for Ahmednagar district (Fig. 4). Boxes indicate the 25<sup>th</sup> to 75<sup>th</sup> percentile range, bands in the middle represent the median, and lower and upper bands indicate minimum and maximum respectively and the square inside the box displays arithmetic mean. The

box plot revealed higher degree of variability in the sub-indices of S-SI and E-ACI as compared to E-SI and S-ACI among the 14 tehsils of Ahmednagar.

### Key Drivers and Buffers

In the identified *highly* vulnerable tehsils, the indicators with significantly high index values which *drive up the sensitivity and lower the adaptive capacity* disproportionately, were identified and designated as DRIVERS for targeted interventions. Obviously, BUFFERS are the variables that contribute significantly to a *higher* adaptive capacity and *lower* sensitivity index values. Further investigation of various sub-indicators (22) in such tehsils aided in identifying the drivers (> 0.50 for sensitivity indicators, ≤ 0.50 for adaptive capacity indicators) and buffers (≤ 0.50 for sensitivity indicators, > 0.50 for adaptive capacity indicators) of vulnerability in the Ahmednagar district. High sensitivity in some tehsils of Ahmednagar was influenced by five drivers: (longer) distance to the nearest town (DNT-0.96) and the nearest hospital (DNH-0.78), (higher) proportion of small and marginal farmers (51%), (larger) net sown area (74%), and (low) rainfall (474 mm). (Better) education status (70%) and (better) community infrastructure (78%) were the buffers that contributed to enhancing adaptive capacity in Ahmednagar district.

The one-way ANOVA was used to determine whether there were significant differences across tehsils in the mean values of each indicator. The results revealed that the mean values of all the socio-economic indicators vary significantly across the tehsils ( $p < 0.05$ ). A post-hoc test was carried out to determine which tehsil(s) index value differs significantly from which tehsil(s) of the district. For example, it was found that the mean SI, ACI, and SEVI values of Akola tehsil differed significantly from other tehsils due to the high population of SC/ST (67%), larger share of NSA owned by SMF (38%), and DNR (22%).

### SEVI decision matrix

By graphing SI-R (Sensitivity Index- Rescaled) against ACI-R (Adaptive Capacity Index- Rescaled) values for each tehsil, a 2D decision matrix (Fig. 5) was created to identify socio-economically vulnerable areas. Low vulnerability tehsils were identified as those in the quadrant with SI-R ≤



0.50 and  $ACI-R > 0.50$ . Tehsils with  $SI-R > 0.50$  and  $ACI-R \leq 0.50$  were categorized as having high socio-economic vulnerability. Out of 14 tehsils of Ahmednagar, 5 tehsils (Nevasa, Shrirampur, Shevgaon, Rahuri, and Jamkhed) were found highly vulnerable with rescaled sensitivity  $SI-R > 0.50$  and rescaled adaptive capacity  $ACI-R \leq 0.50$ . Parner, Rahta, and Sangamner were the least vulnerable tehsils.

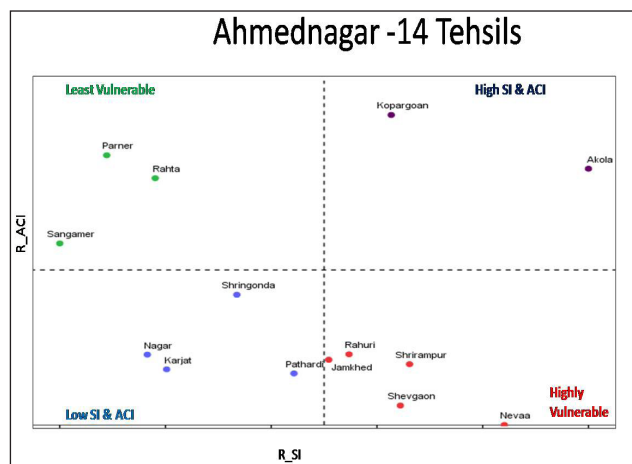


Fig. 5: Tehsil wise decision matrix of Ahmednagar

### Suggestions to reduce socio-economic vulnerability

Only 32% of households in Ahmednagar have proper household amenities, which *Jal Jeevan Mission* can improve by increasing drinking water tap connections to these rural tehsils and providing electricity to unelectrified households through the *Pradhan Mantri Sahaj Bijli Har Ghar Yojana – Saubhagya*. Only about 15% of urban areas in Ahmednagar have access to proper transportation and communication facilities, so the National Broadband Mission can be harnessed to expand internet access to these rural areas, and the *Pradhan Mantri Gram Sadak Yojana* can improve road connectivity to boost these districts' adaptive capacity. Diversification training for livestock can be used to a variety of groups rather than being restricted to a single group. These developments may aid in reducing vulnerability in the Ahmednagar district and raising infrastructure facilities of the district. The study has reaffirmed the importance and utility of assessing and strategizing at the meso- (district) and micro- (tehsil) levels to address socio-economic vulnerability.

### Key interventions to reduce socio-economic vulnerability

The following 7 indicators have been identified as key drivers responsible for high sensitivity and low adaptive capacity in identified tehsils in Ahmednagar district. Specific and targeted interventions corresponding to the identified drivers are narrated for consideration of the development departments and agencies at the tehsil, district and state level (Table 4).

Table 4: Interventions to reduce SI and Increase ACI

	Key drivers	Key intervention/ policy measures
To reduce SI	Small and marginal farmers	Encouraging and mobilising the small and marginal farmers in Nevaa, Rahuri, Rahta, Shrirampur, Shevgaon tehsils to form a collective like Farmer Producer Organisations (FPOs) to overcome the limitations of small scale farm.
	Distance to nearest town	The local governing institutions (Gram and Block Panchayats) in the 14 tehsils of Ahmednagar can take steps to strengthen road connectivity/ infrastructure that can reduce the travel distance significantly.
	Distance to nearest hospital	Explore the feasibility of establishing Primary Health Centres in the remote rural locations in Pathardi, Shringonda, Shevgaon tehsils within a distance of 5 kms to villages.
To improve ACI	Household amenities and housing conditions	Housing and household amenities is a function of household income, availability and accessibility, and awareness and education/attitude. Ensure universal availability and accessibility to basic amenities (sanitation, drinking water, electricity, and cooking fuel) and create adequate awareness in Pathardi, Jamkhed, Karjat, Akola, Shevgaon tehsils.
	Transport and communication	Through Pradhan Mantri Gram Sadak Yojana, strengthen and develop public transportation facilities and access to high speed internet in all the 14 tehsils to improve their coping strategies
	Access to market	Time taken to reach the market can be shortened by better road and public transport, which are very poor as of now in Jamkhed, Akola, Karjat, and Pathardi tehsils.
	Livestock population	This indicator is a measure of household livelihood (income) diversification, which appears to be poor in all the 14 tehsils. The options such as backyard poultry, sheep and duck rearing are feasible and viable, and can supplement household income while also cushioning uncertainties and mitigating dangers.



## CONCLUSION

Ahmednagar is one of Maharashtra's most populous and agriculturally progressive districts with a relatively high human development and less socio-economic vulnerability overall (SEVI score: 0.30). However, tehsil level socio-economic vulnerability assessment highlighted that out of 14 tehsils of Ahmednagar district, 5 tehsils (Nevasa, Shirampur, Shevgaon, Rahuri, and Jamkhed) were found to have high vulnerability levels (sensitivity >0.5 and adaptive capacity <0.5), indicating sub-district level disparities. This study identified 7 key drivers, Small and marginal farmers, Distance to nearest town, Distance to nearest hospital, Household amenities and housing conditions, Transport and communication, Access to market, and Livestock population, that contributed to higher sensitivity and or lower adaptive capacity in few of the tehsils thus leading to overall higher vulnerability levels in those tehsils. The indicator-specific evidence and suggested interventions can be further fine-tuned through coordinated efforts and effectively implemented to reduce sensitivity and increase adaptive capacity in highly vulnerable tehsils. The study also has demonstrated the utility of the SEVI framework in assessing and planning for socio-economic development at the grassroots level. This study recommends conducting a village-level study using the robust SEVI framework to provide even more precise location-specific interventions in highly vulnerable tehsils.

## ACKNOWLEDGEMENTS

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# Effect of Organic Manures on Germination and Growth of Snake Gourd (*Trichosanthes cucumerina* L.)

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## ABSTRACT

The study on "Effect of organic manures on germination and growth of snake gourd (*Trichosanthes cucumerina* L.)" was conducted in Sithalavai village, Karur District, Tamil Nadu during August - November 2021. A field experiment was conducted to study the effect of organic manures on germination and growth of Snake gourd. The organic manures like farm yard manure, vermicompost, phosphobacteria were applied as basal form. The experiment was laid out in randomized block design (RBD) with six treatments, replicated thrice. The results of the investigation revealed that the growth parameters like number of days taken for germination, vine length (cm) and leaf area (cm<sup>2</sup>) were recorded to be highest in the treatment T<sub>4</sub> receiving vermicompost @5t ha<sup>-1</sup> along with RDF, followed by T<sub>6</sub> (RDF + phosphobacteria (2 kg ha<sup>-1</sup>)). The minimum values of all these characters were recorded under control.

## HIGHLIGHTS

- Application of organic manures reduced the days taken for germination in snake gourd.
- Application of organic manures increased the vine length and leaf area thereby increasing the yield of snake gourd.

**Keywords:** Snake gourd, Vermicompost, Farmyard manure, Phosphobacteria, Growth

Vegetables are rich sources of minerals and vitamins for human diet. With increasing population there is an increasing demand of vegetables throughout the world urging a great necessity for increasing the production of vegetables. India prides itself as the second largest producer of vegetables in the world next to China and has maintained a measured growth in production for meeting the growing demands at home and abroad. Among different vegetables, Snake gourd (*Trichosanthes cucumerina* L.) is an important annual cucurbit vegetable, belongs to the family Cucurbitaceae. This annual vine is native of India / Indo Malaya region / the Indian Archipelago (Khatun *et al.* 2010). The plant is regarded as blood purifier and used in curing skin diseases. The traditional method of farming and less use of organic manure reduces the quality of snake gourd. For increasing the productivity economical

fertilizer package need to be formulated which can provide all the essential elements through both organic and inorganic sources to get good quality fruits, produce with higher production, keeping the production cost at sustainable level of an average farmer. Intensive use of only chemical fertilizers to achieve high production has created various problems. Continuous applications of heavy doses of chemical fertilizers without organic manures has led to deterioration of soil health in terms of physical and chemical properties of soil, decrease in soil microbial activities, and also reductions in soil humus (Anjanappa *et al.* 2011). Organic matter plays

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a key role to achieve sustainability on agricultural production because it possesses many desirable properties such as high water holding capacity, cations exchange capacity, beneficial effect on the physical, chemical and biological characteristics of soil. It also adds organic matter to the soil which may improve soil structure, aeration, soil moisture holding capacity and water infiltration (Sundararasu 2017). The use of organic manures like Vermicompost, FYM and Phosphobacteria which have high potentiality to uplift the yield of the crop and favour farmer income. Thus, an experiment was conducted to study the role of organic manures on germination and growth characters of snake gourd.

## MATERIALS AND METHODS

The experiment on “Effect of organic manures on germination and growth of snake gourd (*Trichosanthes cucumerina* L.)” was carried out in the Sitalavai village, Krishnarayapuram block, Karur district located at 10.96° North latitude, 78.28° East longitude at an altitude of 98 m above the mean seal level. The experiment was carried out during 2021-2022. The organic manures were incorporated into soil before sowing along with the recommended dose of fertilizers. Snake gourd variety selected for study was Co-2. The seeds were sown at a distance of 2 × 1.5 m. The cultural operations *viz.*, field preparation, application of manures and fertilizers, Staking and trellising, Irrigation and plant protection were carried out as per the requirement of the crop. The observations recorded for number of days taken for germination, vine length (cm) and leaf area (cm<sup>2</sup>). The data was recorded by taking five plants from each plot which was selected randomly. The statistical analysis of data was done by using DSAASTAT. For treatments showing significance, critical differences were worked out at five percent probability level.

### Treatment details

Treatments	
T <sub>1</sub>	RDF + FYM (10 t ha <sup>-1</sup> ) - Control
T <sub>2</sub>	RDF + FYM (12.5 t ha <sup>-1</sup> )
T <sub>3</sub>	RDF + Vermicompost (2.5 t ha <sup>-1</sup> )
T <sub>4</sub>	RDF + Vermicompost (5 t ha <sup>-1</sup> )
T <sub>5</sub>	RDF + Phosphobacteria (1 kg ha <sup>-1</sup> )
T <sub>6</sub>	RDF + Phosphobacteria (2 kg ha <sup>-1</sup> )

## RESULTS AND DISCUSSION

Basal application of organic manures has shown significant influence on the germination and growth attributes of snake gourd (Table 1). Among the various treatments tested, the minimum days taken for germination was observed in the treatment T<sub>4</sub> (RDF + Vermicompost @5t ha<sup>-1</sup>) with 7.12 days followed by T<sub>6</sub> (RDF + Phosphobacteria @2kg ha<sup>-1</sup>) with 7.63 days. The maximum days taken for germination was observed in T<sub>1</sub> (RDF + FYM @10t ha<sup>-1</sup> - control) with 9.27 days. The vine length was found to be highest (172.23 cm, 556.23 cm and 823.42 cm at 30, 60 DAS and at harvest respectively) in the treatment T<sub>4</sub> which received the application of RDF + Vermicompost @5t ha<sup>-1</sup>. This was followed by the treatment T<sub>6</sub> (RDF + Phosphobacteria @2 kg ha<sup>-1</sup>) and the least value of vine length was observed in T<sub>1</sub> (RDF + FYM @10t ha<sup>-1</sup> - control). The leaf area was found to be maximum in T<sub>4</sub> (RDF + Vermicompost @5t ha<sup>-1</sup>) with 193.92 cm<sup>2</sup> followed by T<sub>6</sub> (RDF + Phosphobacteria @2kg ha<sup>-1</sup>) with 175.05 cm<sup>2</sup> and least leaf area were noted in T<sub>1</sub> (RDF + FYM @10t ha<sup>-1</sup> - control) with 106.76 cm<sup>2</sup> (Table 2).

Vermicompost which would have improved the physical properties of the soil, such as they would have provided more nitrogen and phosphorous in the soil. Organic manures improve the soil physical conditions and improves microbial and soil organic matter which in turn produces organic acids which inhibits particularly IAA oxidase enzymes, resulting in enhancing the promotive effect of auxins which has direct effect on plant (Hammad *et al.* 2011). The early germination may be due to high humidity and warming up of the soil created by organic matter supplied (Rabari *et al.* 2019). These results are in consistent with the findings of Parmar *et al.* (2011) in cucumber, Sarhan *et al.* (2011) in summer squash, Antoinette *et al.* (2013) in okra and Enujeke *et al.* (2013) in watermelon. The increase in vine length might be due to vermicompost which would have improved the physical properties of the soil, such as they would have provided more nitrogen and phosphorous in the soil (Sureshkumar *et al.* 2019). Due to the presence of diverse amount of nutrients in vermicompost, they improved growth of the plant at lower concentration and inhibited growth at higher concentration with similar physiological effects to that of phytohormones (Provasoli and

**Table 1:** Mean values on the effect of organic manures and biostimulants over days taken for germination and vine length of snake gourd

Tr. No.	Treatment details	Number of days taken for germination	Vine length (cm)		
			30 DAS	60 DAS	Har
T <sub>1</sub>	Control - RDF + FYM (10 t ha <sup>-1</sup> )	9.27	108.13	357.76	470.63
T <sub>2</sub>	RDF + FYM (12.5 t ha <sup>-1</sup> )	8.83	117.52	399.33	523.86
T <sub>3</sub>	RDF + Vermicompost (2.5 t ha <sup>-1</sup> )	8.66	141.70	460.28	626.75
T <sub>4</sub>	RDF + Vermicompost (5 t ha <sup>-1</sup> )	7.12	172.23	556.23	823.42
T <sub>5</sub>	RDF + Phosphobacteria (1 kg ha <sup>-1</sup> )	8.76	129.97	431.92	570.41
T <sub>6</sub>	RDF + Phosphobacteria (2 kg ha <sup>-1</sup> )	7.63	162.10	523.63	753.06
	S.ED	0.14	2.68	8.63	12.36
	CD (p=0.05)	0.31	5.97	19.22	27.54

**Table 2:** Mean values on the effect of organic manures and biostimulants on leaf area of snake gourd

Tr. No.	Treatment details	Leaf area (cm <sup>2</sup> )		
		30 DAS	60 DAS	Har
T <sub>1</sub>	Control - RDF + FYM (10 t ha <sup>-1</sup> )	68.39	99.65	106.76
T <sub>2</sub>	RDF + FYM (12.5 t ha <sup>-1</sup> )	71.42	108.52	126.15
T <sub>3</sub>	RDF + Vermicompost (2.5 t ha <sup>-1</sup> )	79.99	129.21	161.62
T <sub>4</sub>	RDF + Vermicompost (5 t ha <sup>-1</sup> )	90.17	138.42	193.92
T <sub>5</sub>	RDF + Phosphobacteria (1 kg ha <sup>-1</sup> )	75.15	118.78	148.33
T <sub>6</sub>	RDF + Phosphobacteria (2 kg ha <sup>-1</sup> )	84.31	133.88	175.05
	S.ED	1.44	2.31	3.07
	CD (p=0.05)	3.22	5.14	6.84

Carlucci, 1974). The result of the present study is in agreement with the findings of Singh *et al.* (2012), Das *et al.* (2015), Singh *et al.* (2017) and Patle *et al.* (2018) in bottle gourd, Sangeetha *et al.* (2018) in bitter gourd, Baghel *et al.* (2017) and Rathod *et al.* (2018) in ridge gourd. The significant increase in leaf area might be due to better nutrient supply by specific combination of INM treatments which leads to increase the internal metabolic activities in plants (Singh *et al.* 2020). Further, Dalorima *et al.* (2018) reported that vermicompost improves the soil physical and chemical properties and gives crop optimum conditions for growth and development of watermelon. Higher rates of manure might have improved soil properties and increase the growth and development of snake gourd. The similar results were also reported by Anjanappa *et al.* (2012) in cucumber and Sureshkumar *et al.* (2019) in bitter gourd.

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# Comparative Analysis of Performance of Different Fodder Crops Under Pigeonpea Based Intercropping System (1:2)

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## ABSTRACT

India stands first in milk production in the world, but average milk yield is very low (5 litres per animal) compared to developed countries (24 litres per animal). Deficit of green fodder is one of the main reasons for low milk yield along with other factors like imbalanced nutrition, good quality fodder *etc.* In pigeonpea growing areas of north Karnataka there is an acute shortage of fodder crops is faced. Most of the farmers depend on the pigeonpea for their livelihood. But, as fodder shortage has become one of the major problems of pigeonpea growing area. There is a need to grow fodder crops without affecting the yield of pigeonpea. Hence, improving cropping system can be a probable solution. Intercropping short duration fodder crops in pigeonpea may yield good fodder yields as well as grain yields.

## HIGHLIGHTS

- ① Reducing fodder scarcity problems by adapting intercropping systems
- ① Efficient utilisation of area, time, space and other resources under intercropping system despite higher productivity and profitability.

**Keywords:** Cropping system, fodder, intercropping and pigeonpea

Forages are the back bone of livestock industry. The foundation of animal health and their production depends on availability of fodder. The scarcity of green forages and grazing resources in the country has made livestock to suffer from malnutrition resulted in decreasing production potentiality at below average compared to many developed countries. India has the largest livestock population, which accounts for 17.5 per cent of the world's livestock population. However, livestock productivity is constrained by an acute shortage of feed and fodder. In India, due to increased population pressure and competition from the food crops for natural resources like land, water, sunlight *etc.*, therefore it is not possible to increase the area under fodder crops further. The only way to bridge the large gap between fodder demand and

supply of fodder is through maximizing the fodder production per unit area and unit time. The shortfall can be met by improving the cropping systems and increasing the cropping intensity.

Pigeonpea is the major crop of northern parts of Karnataka, being a long duration and widely spaced crop offers ample opportunity for intercropping. Cultivating fodder crops as intercrop in pigeonpea helps in reducing the fodder scarcity problems in pigeonpea growing areas and better livestock development. In this regard the present investigation

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was undertaken to study the performance of fodder crops under intercropping system with pigeonpea.

## MATERIALS AND METHODS

A field experiment was conducted to study the performance of different fodder crops under pigeonpea based intercropping system. There were 13 treatments replicated thrice. Different fodder crops such as fodder sorghum, fodder maize, fodder bajra, fodder cowpea, fodder horsegram and fodder fieldbean were intercropped with pigeonpea at 1:2 row proportions. And each fodder crop was compared with the sole fodder crop.

## RESULTS AND DISCUSSION

### Growth parameters

The results of the experiment clearly indicated that all the growth parameters *viz.*, plant height, leaf area, leaf area index and total dry matter accumulation of fodder crops was considerably influenced by intercropping with pigeonpea. All the sole fodder crops recorded considerably higher plant height, leaf area, leaf area index and total dry matter accumulation than the intercropped fodder crops. This may be due to the less competition in sole cropping. The results of the experiment are in conformity with the results of Singh and Jadhav (2003) in Sorghum + pigeonpea intercropping

system. Prajapati *et al.* (2018) and Patel *et al.* (2018) also recorded similar results.

### Green fodder yield

Considerable difference in green fodder yield of fodder crops was recorded when intercropped with pigeonpea. Among all the cereal fodder crops fodder maize shown considerably higher green fodder yield over others. Green fodder yield of fodder maize in sole cropping ( $T_3$ ) ( $17.28 \text{ t ha}^{-1}$ ) was considerably higher than the green fodder yield of fodder maize intercropped with pigeonpea ( $T_9$ ) ( $12.24 \text{ t ha}^{-1}$ ).

Among all the legume fodder crops, fodder horsegram had shown considerably higher green fodder yield over others. Green fodder yield of fodder horsegram in sole cropping ( $T_6$ ) ( $18.60 \text{ t ha}^{-1}$ ) was considerably higher than the green fodder yield of fodder horsegram intercropped with pigeonpea ( $T_{12}$ ) ( $12.96 \text{ t ha}^{-1}$ ).

Experimental results indicated that the sole crop yield of all the fodder crops were considerably higher than the intercropped fodder crops. This may be due to the increased competition for light, moisture, space and nutrients in the intercropping system. Similar results were obtained by Surve *et al.* (2011) they reported that sole fodder cowpea recorded considerably higher green fodder yield compared to maize + fodder cowpea and sorghum

**Table 1:** Plant height, leaf area, leaf area index (LAI), Total dry matter production (TDMP) of different fodder crops at 45 days after sowing under pigeonpea based fodder intercropping system

Treatments	Plant height (cm)	Leaf area ( $\text{cm}^2 \text{ plant}^{-1}$ )	LAI	TDMP ( $\text{g plant}^{-1}$ )
$T_1$ - Sole Pigeonpea	—	—	—	—
$T_2$ - Sole Fodder Sorghum	55.6	7.25	2.42	24.47
$T_3$ - Sole Fodder Maize	83.2	11.66	3.89	32.80
$T_4$ - Sole Fodder Bajra	75.7	8.00	2.67	25.07
$T_5$ - Sole Fodder Cowpea	48.6	5.02	1.67	15.03
$T_6$ - Sole Fodder Horsegram	50.6	5.25	1.75	15.25
$T_7$ - Sole Fodder Fieldbean	48.8	4.75	1.58	14.08
$T_8$ - Pigeonpea + Fodder Sorghum (1:2)	51.0	5.88	1.96	22.80
$T_9$ - Pigeonpea + Fodder Maize (1:2)	78.0	9.95	3.32	31.70
$T_{10}$ - Pigeonpea + Fodder Bajra (1:2)	70.0	6.25	2.08	23.13
$T_{11}$ - Pigeonpea + Fodder Cowpea (1:2)	43.6	4.50	1.50	12.92
$T_{12}$ - Pigeonpea + Fodder Horsegram (1:2)	45.6	4.81	1.60	13.58
$T_{13}$ - Pigeonpea + Fodder Fieldbean (1:2)	44.0	4.40	1.47	12.42

**Table 2:** Plant height, leaf area, leaf area index (LAI), Total dry matter production (TDMP) of different fodder crops at harvest under pigeonpea based fodder intercropping system

Treatments	Plant height (cm)	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	LAI	TDMP (g plant <sup>-1</sup> )
T <sub>1</sub> - Sole Pigeonpea	—	—	—	—
T <sub>2</sub> - Sole Fodder Sorghum	71.2	8.92	2.97	29.47
T <sub>3</sub> - Sole Fodder Maize	102.3	13.35	4.45	35.57
T <sub>4</sub> - Sole Fodder Bajra	87.7	9.17	3.06	30.03
T <sub>5</sub> - Sole Fodder Cowpea	58.0	6.73	2.24	19.17
T <sub>6</sub> - Sole Fodder Horsegram	59.2	7.18	2.39	21.27
T <sub>7</sub> - Sole Fodder Fieldbean	58.3	6.42	2.14	18.17
T <sub>8</sub> - Pigeonpea + Fodder Sorghum (1:2)	67.0	7.33	2.44	26.80
T <sub>9</sub> - Pigeonpea + Fodder Maize (1:2)	92.4	11.56	3.85	32.43
T <sub>10</sub> - Pigeonpea + Fodder Bajra (1:2)	80.3	7.92	2.64	27.63
T <sub>11</sub> - Pigeonpea + Fodder Cowpea (1:2)	53.0	5.27	1.76	17.67
T <sub>12</sub> - Pigeonpea + Fodder Horsegram (1:2)	55.5	5.77	1.92	18.33
T <sub>13</sub> - Pigeonpea + Fodder Fieldbean (1:2)	55.2	5.08	1.69	16.60

**Table 3:** Green fodder yield of different fodder crops as influenced by intercropping with pigeonpea

Treatments	Green fodder yield (t ha <sup>-1</sup> )
T <sub>1</sub> - Sole Pigeonpea	—
T <sub>2</sub> - Sole Fodder Sorghum	14.06
T <sub>3</sub> - Sole Fodder Maize	17.28
T <sub>4</sub> - Sole Fodder Bajra	15.26
T <sub>5</sub> - Sole Fodder Cowpea	13.45
T <sub>6</sub> - Sole Fodder Horsegram	18.60
T <sub>7</sub> - Sole Fodder Fieldbean	13.05
T <sub>8</sub> - Pigeonpea + Fodder Sorghum (1:2)	9.71
T <sub>9</sub> - Pigeonpea + Fodder Maize (1:2)	12.24
T <sub>10</sub> - Pigeonpea + Fodder Bajra (1:2)	10.36
T <sub>11</sub> - Pigeonpea + Fodder Cowpea (1:2)	9.02
T <sub>12</sub> - Pigeonpea + Fodder Horsegram (1:2)	12.96
T <sub>13</sub> - Pigeonpea + Fodder Fieldbean (1:2)	8.73

+ fodder cowpea intercropping systems. Lingaraju *et al.* (2008) and Sharma *et al.* (2008) also recorded similar results

## CONCLUSION

Sole crop performance of all the fodder crops was considerably better than the intercropped fodder crops. This may be due to competition for resources in intercropping system. However, intercropping systems found to be more profitable despite of recording lower green fodder yields because, in intercropping system in addition to green fodder yields, pigeonpea grain yield was produced. Effective utilisation of light, moisture, space and

time is done. Among different fodder crops, Fodder horsegram is found to be best intercrop since it recorded higher green fodder yield.

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# Effect of Subsurface Drainage on TSS and SAR in Saline Vertisol Under TBP Command Area

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## ABSTRACT

A study was conducted to ensure the impact of subsurface drainage system on salt affected soils in the TBP command area, Karnataka. The mean values of Total Soluble Salts (TSS) and Sodium Adsorption Ratio (SAR) of pre-drainage soil samples was 3.82 to 6.32, and 8.23 to 14.41 respectively, while it was reduced in post-drainage soil samples *i.e.*, 2.30 to 4.90 and 5.46 to 12.19 for TSS and SAR respectively. The concentration of TSS and SAR of post-drainage soil samples was reduced in all the depths than that of corresponding depths in pre-drainage soil samples by leaching effect and draining of water from the field, thus indicating the positive effect of SSD in removal of salts throughout the profile soil depth.

## HIGHLIGHTS

- Installation of SSD has positively impacted the soil through significant leach out of soluble salts more effectively in surface soil depths.

**Keywords:** Subsurface drainage system, Total soluble salts and Sodium adsorption ratio

Introduction of irrigated schemes in arid and semi-arid regions of the country has led to the occurrence of twin problems *i.e.*, water logging and soil salinization owing to inadequate drainage system, thus a considerable area either going out of production or experiencing reduced yield. Apart from this, the increased use of saline water together with poor management practices aggravated the problem, particularly when there is inadequate drainage of water. As the water table rises, salts stored within the soil profile are mobilized and carried towards the soil surface, resulting in salinization. The reclamation of these salt affected soils requires an efficient technique to decrease the salinity in the upper soil layer for improved root growth and crop development. Among the several reclamations measures the subsurface drainage technology has been widely used for reclamation

of water logged and salt affected lands in many parts of India. Subsurface drainage describes the process of removal of that water which has infiltrated into the soil in excess of the amount that can be held by capillary forces against the force of gravity (Kapourechal *et al.* 2013). Soils that require accelerated subsurface drainage typically have some impermeable or slowly permeable feature below the surface that prevents water that has entered the soil from moving deeper into the soil and underlying materials at a rate that allows agricultural production to be economically

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viable. Therefore, in order to tackle the problem of water logging and soil salinity and to reclaim the affected soils TBP CADA has planned to carry out the reclamation work in a phased manner. Under phase-IV, it has initiated work in an area of 4080.64 ha distributed under four sub divisions.

## MATERIALS AND METHODS

### Study Area and Climate

The study area selected for the present study comes under the Tungabhadra command area and project site is situated at a distance of 21.0 km on Ballari to Gotur road and it is 2.00 km from the Gotur village with 15°13'93.93" N latitude and 76°92'14.43" E longitude at a elevation of 495 m above the mean sea level. A block of 80 ha area comprising of different farmers' fields has been selected where in the subsurface drainage system was implemented during 2016. The study area falls under the Northern Dry Zone (Zone-2) of Karnataka State Agro-climatic Zones Classification. The area is a part of semi-arid region characterized by mild winter, short monsoon and hot summer. The mean annual temperature is 27.4 °C. Summer season is very hot with temperatures rising to 42 °C or more, whereas winter season (November to February) is relatively cool and dry. The hottest months are April and May, and December is the coldest month. The average annual rainfall at Ballari rain gauge station is 550.16 mm, of which 350.6 mm occurs during June-September, which is 62.26 per cent of the average annual rainfall.

### Collection and preparation of soil samples for chemical analysis

In order to carry out systematic studies, the sampling points were identified on a grid size of 50 m × 50 m in the study area (9 points). The soil samples were collected at different depths of 0-30, 30-60, 60-90 and 90-120 cm from each grid points during 2016 before the installation of subsurface drainage. The post subsurface drainage soil samples were collected after the harvest of first crop *i.e.*, during 2017. However, care was taken to keep the soil sampling points as same as those of pre-drainage points using GPS. The soil samples were air dried in the shade, ground with wooden pestle and mortar and passed through 2 mm sieve.

Samples were preserved in polyethylene bags for further chemical analysis. The chemical analysis included estimating water soiluble cations and anions *i.e.*, Ca<sup>+</sup>, Mg, Na, K, Cl, SO<sub>4</sub><sup>2-</sup> and HCO<sub>3</sub><sup>-</sup>. The comparison of soil parameters of pre SSD and post SSD soil samples was carried out by using paired t-test and impact of SSD was assessed.

### Soil chemical properties

#### Sodium adsorption ratio (SAR)

The sodium adsorption ratio of the soil was computed using the below formula:

$$SAR = \frac{Na^+}{\sqrt{(Ca^{2+} + Mg^{2+})/2}}$$

Where, Na, Ca and Mg are the concentrations in saturation extraction expressed as meq per litre.

#### Total soluble salts (%)

The total soluble salts in saturation extract of soil samples is estimated by using the below formula:

$$TSS (\%) = EC_e \times \frac{640}{10000}$$

Where, EC<sub>e</sub> is in dS m<sup>-1</sup>

## RESULTS AND DISCUSSION

### Sodium adsorption ratio (SAR) and Total soluble salts percent (TSS)

The analyses results pertaining to SAR and TSS (%) of pre and post drainage soil samples are presented in Table 1. In general, among the pre-drainage soil samples the SAR values ranged from 8.23 to 14.41 while, TSS (%) values ranged from 3.82 to 6.32 irrespective of soil depths. The results revealed that the mean values for SAR of pre-drainage soil samples drawn from 0-30, 30-60, 60-90 and 90-120 cm soil depths were 10.31, 10.29, 11.52 and 10.90 respectively and results were not showing any definite trend with an increase in depth of the soil under study. While the mean TSS values were 5.26, 5.02, 4.67 and 4.35 per cent respectively, showing a slight decrease with the soil depth (Table 1).

On the other hand, in the case of post SSD soil samples, in general the SAR values ranged from

**Table 1:** Effect of SSD on sodium adsorption ratio (SAR) and total soluble salts (%) recorded in soil samples collected from different sampling points

Soil depth (cm)	SAR			TSS		
	(a) Pre-drainage					
	Min.	Max.	Mean	Min.	Max.	Mean
0-30	9.21	12.10	10.31	4.83	6.32	5.26
30-60	8.53	12.50	10.29	4.51	5.69	5.02
60-90	8.81	14.41	11.52	4.30	5.25	4.67
90-120	8.23	13.73	10.90	3.82	4.83	4.35
(b) Post-drainage						
0-30	5.46	9.92	7.90	2.30	3.71	3.24
30-60	6.55	10.65	8.24	3.62	4.27	3.86
60-90	7.46	12.19	9.17	3.70	4.77	4.24
90-120	7.97	12.09	9.37	4.13	4.90	4.43

**Table 2:** Comparison of SAR and TSS of pre-drainage and post-drainage soil samples using paired t-test

Soil depth (cm)	SAR		TSS	
	$t_{cal}$	$t_{cri}$	$t_{cal}$	$t_{cri}$
0-30	6.24*		12.87*	
30-60	4.26*	1.86*	8.31*	1.86*
60-90	4.09*		6.23*	
90-120	3.05*		-0.39	

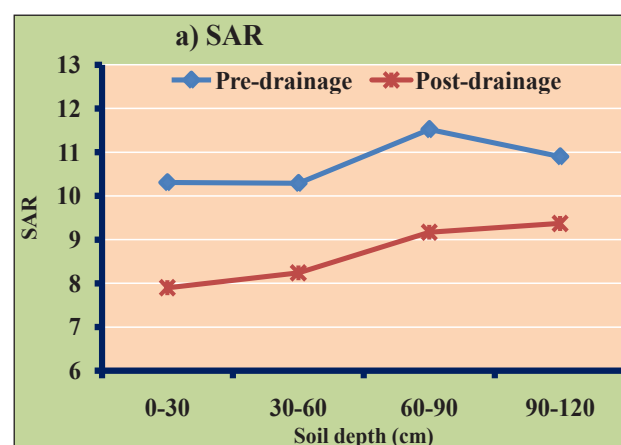
5.46 to 12.19 while TSS (%) values ranged from 2.30 to 4.90 irrespective of soil depths. While the analyses of soil samples from post SSD work have indicated a decrease in both mean values of SAR and TSS (%) in all soil depths, except in 90-120 cm for TSS (%) *i.e.*, 7.90, 8.24, 9.17, 9.37 and 3.24, 3.86, 4.24, 4.43 for 0-30, 30-60, 60-90 and 90-120 cm soil depths respectively (Table 1).

The comparative analysis of SAR and TSS (%) content of pre and post-drainage soil samples using paired t test (Table 1) revealed that SSD had significantly impacted and decreased the soil SAR in all the depths while the decrease in soil TSS was significant in all the depths with the exception of 90-120 cm which had comparatively higher TSS than the pre SSD but the increase was non-significant (Table 1).

The SSD positively impacted by reducing the sodium adsorption ratio (Fig. 1) and total soluble salts of post-drainage soil samples in all the depths than SAR and TSS of corresponding depths of pre-drainage soil samples with the exception of 90-120 cm soil depth for TSS wherein salts got accumulated more when compared to pre-drainage soil samples

.The reduction in SAR and TSS of post-drainage soil samples is due to the leaching and accumulation of salts in lower depths. These results are in conformation with the findings of Doddamani *et al.* (1995), Anand (2003) and Patil *et al.* (2016) wherein they observed the decrease in SAR values after the installation of subsurface drainage.

The distribution of surface soil SAR in the entire study area before and after SSD is depicted in the map (Fig. 1).

**Fig. 1:** Influence of SSD on soil SAR observed at different soil depth

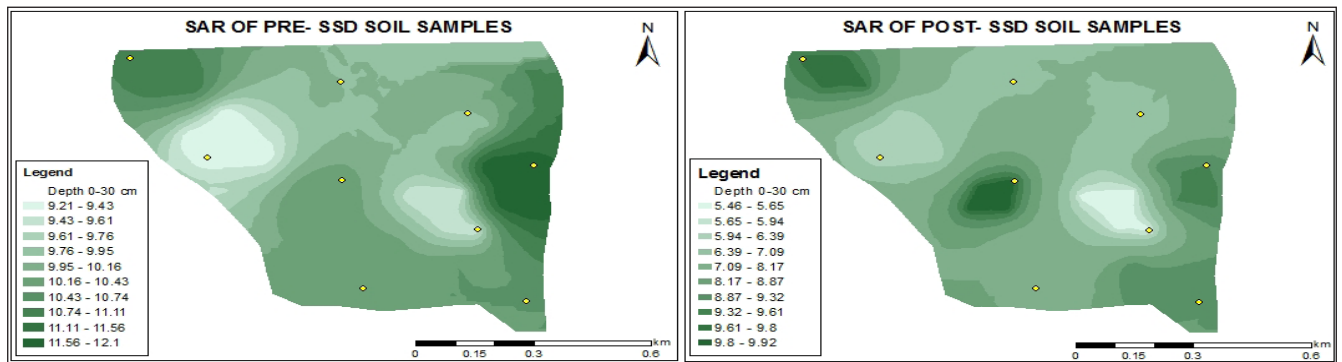


Fig. 2: Map showing variation in SAR of pre and post-drainage soil samples of study

## CONCLUSION

The provision of SSD positively impacted soil properties such as sodium adsorption ratio and total soluble salts of post-drainage soil samples in all the depths by leaching of water soluble salts.

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# Genetic Variability of Determinate F<sub>4</sub> Progenies for Yield Attributes of Indian Bean [*Lablab purpureus* (L.) Sweet]

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## ABSTRACT

The experiment was conducted with fifty-five F<sub>4</sub> progenies along with two checks of Indian bean (*Lablab purpureus* L. Syn. *Dolichos lablab* L., 2n=22) to evaluate genetic variability among eleven characters during late *Kharif* season, 2018-19 at Navsari Agricultural University, Gujarat. Significant variation was observed among all the traits investigated except days to maturity and pod length as well as within progenies of cross B, cross C and cross D. Seed yield per plant, pods per plant, racemes per plant, pods per raceme, pod weight and pod width showed high estimates of GCV and PCV. High heritability coupled with high genetic advance expressed as percentage of mean was noted for pod width and pod weight showing role of additive gene action and less effect of environment. Seed yield per plant had positive and highly significant association with days to maturity (0.850), plant height (0.526), racemes per plant (0.355), pods per raceme (0.460), pods per plant (0.434), pod width (0.552), pod weight (0.698) and seeds per pod (0.207). Also pod weight and pods per plant had higher magnitude of positive direct effect on seed yield per plant and hence, advancement in seed yield could be brought by pod weight and pods per plant.

## HIGHLIGHTS

- ① The experiment was conducted with fifty-five F<sub>4</sub> progenies along with two checks of Indian bean (*Lablab purpureus* L. Syn. *Dolichos lablab* L., 2n=22) to evaluate genetic variability among eleven characters during late *Kharif* season, 2018-19 at Navsari Agricultural University, Gujarat.
- ① Analysis of variance revealed significant variation among all the characters except days to maturity and pod length (Table 1) of progenies.
- ① Significant variation was observed among all the traits investigated except days to maturity and pod length as well as within progenies of cross B, cross C and cross D.
- ① High heritability coupled with high genetic advance expressed as percentage of mean was observed for pod width (82.60%, 42.64%) and pod weight (64.30%, 33.61%).
- ① Pod weight and pods per plant exhibited highly significant and positive association and higher magnitude of positive direct effect on seed yield per plant and hence, these may be considered as most important yield contributing characters and due emphasis should be placed on these components while applying selection for high seed yield in Indian bean.

**Keywords:** Determinate F<sub>4</sub> progenies, Genetic variability, heritability, Genotypic correlation, Genotypic path

Indian bean (*Lablab purpureus* L. Syn. *Dolichos lablab* L., 2n=22) is one of the important legumes as well as vegetable crops. India is its centre of diversity. A wide range of variation exists for the plant and pod characters amongst the accessions grown all over the country. Most of the land races / varieties

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are indeterminate in growth habit as well as photo sensitive. On the other hand, there are very few determinate and photoinensitive races/varieties available for cultivation. Determinate and early cultivars provide option for alternative cropping systems and its diversification, allowing farmers to plant and harvest more.

The variability of different plants is studied by different plant breeding methods. Since, many of the plant characters are governed by polygenes and greatly influenced by environmental conditions; the progress of breeding is, however, conditioned by the magnitude, nature and interrelationship of genotypic and non-genotypic variation.

Genetic parameters such as genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are useful in detecting the amount of variability present among the genotypes studied. Similarly, knowledge of heritability is essential for selection of superior genotypes. It is a good index of the transmission of characters from parents to the offspring (Falconer, 1960). Genetic advance is the difference between the mean genotypic value of the selected lines and the mean genotypic value of the base population.

Yield is a complex character governed by quantitative traits and the environment. Thus, selection for seed yield becomes difficult unless the association between the yield contributing characters are known. The association between seed yield and its component characters can better be interpreted through cause and effect analysis i.e. path coefficient analysis which quantify the direct and indirect influence of one character upon another (Dewey and Lu 1959).

The present study conducted (1) To study variance components, heritability and genetic advance for yield and its components and (2) To estimate correlations and path coefficients for assessing contribution of each of the yield components.

## MATERIALS AND METHODS

The present study was conducted during late *Kharif* season, 2018-19 at the College Farm, N. M. College of Agriculture, Navsari Agricultural University, Gujarat which is geographically situated at 20°37'N latitude and 72°54'E longitude as well as at an altitude of 11.98 meters above mean sea level. The

experimental material was generated carefully choosing the parents with major aim of improving the performance of genotypes for plant height, pods per plant, seeds per pod, racemes per plant and seed yield per plant. The experiment comprised of 55 F<sub>4</sub> progenies obtained from four crosses *viz.*, GNIB-21 × GP-1 (cross A, 2 progenies), GNIB-21 × GP-167 (cross B, 14 progenies), GNIB-21 × GP-189 (cross C, 22 progenies), GNIB-21 × GPKH-120 (cross D, 17 progenies) and two checks GNIB-21 and GNIB-22 of Indian bean. Released variety GNIB-21 was kept as common parent because of its determinate growth habit, early flowering, consumer preference and high harvest index. While male parents have indeterminate growth habit, long duration and photo-period sensitive flowering. Observations on quantitative characters *viz.*, days to 50 per cent flowering (DTF), days to maturity (DTM), plant height (PH), raceme per plant (RPP), pods per raceme (PPR), pods per plant (PPP), pod length (PL), pod width (PW), pod weight (PWT), seeds per pod (SPP) and seed yield per plant (SY) were recorded from parents and their F<sub>4</sub> progenies. The experiment was conducted in Randomized Complete Block Design (RCBD) with three replications. Total area of experimental unit was 650 m<sup>2</sup> (50 m × 13 m). Each row consisted of 15 plants of a progeny with 60 cm × 20 cm inter and intra row spacing. The progenies were randomly allotted to the plots in each replication. The experiment was surrounded by one guard row to avoid damage and border effect. All the recommended agronomical practices along with necessary plant protection measures were followed timely for the successful raising of the crop.

The data recorded were subjected to analysis of variance (Panse and Sukhatme 1978). Phenotypic and genotypic coefficient of variation (Burton, 1953); Heritability in broad sense and Genetic Advance (GA) (Allard 1960); Genetic advance expressed as per cent of mean (Johnson *et al.* 1955); Genotypic ( $r_g$ ) correlation coefficients (Miller *et al.* 1958) and Path analysis (Wright 1921 and Dewey and Lu 1959) were estimated for interpreting the results.

## RESULTS AND DISCUSSION

Analysis of variance revealed significant variation among all the characters except days to maturity and pod length (Table 1) of progenies. Further

**Table 1:** Analysis of variance for quantitative characters in Indian bean

Source of Variation	Replication	Treatment	Crosses	Cross A	Cross B	Cross C	Cross D	Checks	Crosses v/s Check	Error
(df)	(2)	(56)	(3)	(1)	(13)	(21)	(16)	(1)	(1)	(112)
DTF	19.111**	7.481**	18.331**	10.667	8.608**	7.475**	4.581	10.667	0.45	3.194
DM	57.07	19.783	12.222	10.667	17.137	21.608	22.824	0	18.758	19.118
PH	494.463**	151.983**	699.953**	0	168.363**	131.754**	81.739**	1.685	146.119*	36.055
RPP	12.533**	4.669**	2.136	6.242	5.948**	2.682	7.142**	0.341	0.562	1.961
PPR	1.028	1.848**	19.706**	1.27	0.45	0.563	1.421*	0.778	1.921	0.663
PPP	363.097**	75.875**	435.65**	10.747	52.635*	25.456	98.839**	1.904	129.143*	23.746
PL	0.01	0.424	1.603*	0.416	0.328	0.445	0.263	0.023	0.666	0.455
PW	0.009	0.22**	1.805**	0.001	0.16**	0.109**	0.121**	0.029	0.549**	0.013
PWT	0.036	0.078**	0.787**	0.091**	0.066**	0.027**	0.021*	0.006	0.139**	0.012
SPP	0.823**	0.188*	0.569**	0.001	0.131	0.196	0.136	0.001*	0.846	0.129
YPP	49.811**	20.925**	41.144**	22.815	31.925**	11.76*	21.482**	0.24	19.597	6.606

**Table 2:** Genetic variability parameters of quantitative characters in Indian bean

Character	Mean	Range		$\sigma_g^2$	$\sigma_p^2$	$\sigma_e^2$	GCV (%)	PCV (%)	ECV (%)	$h_b^2$ (%)	GA	GA as percent of mean (%)
		Min.	Max.									
Days to 50% flowering	53.40	50.67	58.00	1.42	4.62	3.20	2.24	4.03	3.34	30.90	136.90	2.56
Days to maturity	111.60	108.00	117.00	0.22	19.34	19.12	0.42	3.94	3.91	1.10	10.40	0.09
Plant height (cm)	47.13	25.15	58.95	38.64	74.70	36.06	13.19	18.34	12.74	51.70	921.00	19.54
Racemes per plant	5.71	2.35	10.25	0.90	2.86	1.96	16.64	29.63	24.52	31.50	109.90	19.24
Pods per raceme	3.92	2.26	5.20	0.40	1.06	0.66	16.05	26.27	20.79	37.30	79.10	20.20
Pods per plant	17.51	7.92	32.96	17.38	41.12	23.74	23.81	36.63	27.83	42.30	558.20	31.89
Pod length (cm)	5.63	4.78	6.38	-0.01	0.45	0.46	—	11.84	11.98	—	—	—
Pod width (cm)	1.13	0.71	1.66	0.07	0.08	0.01	22.78	25.06	10.45	82.60	47.90	42.64
Pod weight (g)	0.73	0.42	1.06	0.02	0.03	0.01	20.35	25.38	15.17	64.30	24.50	33.61
Seeds per pod	3.33	2.80	3.82	0.03	0.16	0.13	5.26	12.14	10.94	18.80	15.60	4.69
Seed yield per plant (g)	8.56	3.70	17.18	4.77	11.38	6.61	25.52	39.40	30.02	41.90	291.50	34.04

**Table 3:** Genotypic correlations coefficient analysis for seed yield and its attributes

	DTF	DM	PH	RPP	PPR	PPP	PL	PW	PWT	SPP	SY
DTF	1.000	-0.276**	0.080	0.009	-0.102	-0.193*	-0.258**	0.168*	0.410**	0.030	0.128
DM		1.000	0.301**	-0.201**	-1.216	0.304**	-1.697	-0.501**	0.218**	-0.436**	0.850**
PH			1.000	0.158*	-0.205**	-0.106	-1.590	0.208**	0.678**	0.548**	0.526**
RPP				1.000	0.179*	0.707**	-0.628**	-0.196	-0.086	0.310**	0.355**
PPR					1.000	0.711**	-1.526	0.294**	0.017	0.095	0.460**
PPP						1.000	-0.803**	-0.037	-0.269**	-0.198**	0.434**
PL							1.000	-2.074	-2.255	-1.246	-2.334
PW								1.000	0.719**	0.414**	0.552**
PWT									1.000	0.514**	0.698**
SPP										1.000	0.207**
SY											1.000

**Table 4:** Genotypic path coefficient analysis for seed yield and its attributes

	DTF	DM	PH	RPP	PPR	PPP	PL	PW	PWT	SPP	
DTF	-0.166**	-0.012	-0.017	-0.001	0.008	-0.148	0.010	-0.045	0.500	-0.001	0.128
DM	0.046	0.043*	-0.063	0.019	0.093	0.234	0.066	0.134	0.265	0.013	0.850**
PH	-0.013	0.012	-0.208**	-0.015	0.015	-0.081	0.062	-0.056	0.826	-0.016	0.526**
RPP	-0.001	-0.009	-0.033	-0.097	-0.014	0.545	0.026	0.052	-0.105	-0.009	0.355**
PPR	0.017	-0.052	0.043	-0.017	-0.076*	0.547	0.060	-0.079	0.020	-0.003	0.460**
PPP	0.032	0.013	0.022	-0.068	-0.054	0.770**	0.031	0.010	-0.328	0.006	0.434**
PL	0.043	-0.073	0.331	0.061	0.117	-0.618	-0.039	0.555	-2.747	0.036	-2.334
PW	-0.028	-0.021	-0.043	0.019	-0.023	-0.029	0.081	-0.268**	0.876	-0.012	0.552**
PWT	-0.068	0.009	-0.141	0.008	-0.001	-0.207	0.088	-0.193	1.218**	-0.015	0.698**
SPP	-0.005	-0.019	-0.113	-0.030	-0.007	-0.153	0.049	-0.111	0.626	-0.030	0.207**

\*\* , \*Significant at  $p \leq 0.01$  and  $p \leq 0.05$

GCV = Genotypic coefficient of variation

$h_b^2$  = Heritability (Broad sense)

$\sigma_g^2$  = Genotypic variance

PCV = Phenotypic coefficient of variation

GA = Genetic advance

$\sigma_p^2$  = phenotypic variance

(-) = Negative values were considered as zero for GCV,  $h_{bs}^2$ , GA and GA as per cent of mean

DTF = Days to 50% flowering

DM = Days to maturity

PH = Plant height (cm)

SY = Seed Yield per plant (g)

PPR = Pods per raceme

PWT = Pod weight (g)

PPP = Pods per plant

PW = Pod width (cm)

RPP = Racemes per plant

PL = Pod length (cm)

SPP = Seeds per pod

partitioning showed significant differences among crosses for all traits barring DM and RPP. Progenies within cross A yielded significant variation in PWT; cross B for DTF, PH, RPP, PPP, PW, PWT and YPP; cross C for DTF, PH, PW, PWT and YPP and cross D for PH, RPP, PPP, PW, PWT and YPP. Similar results were reported by Hadavani *et al.* (2018) and Peer *et al.* (2018). Cross A, in general, did not show influence of selection pressure on any attributes studied.

Lower difference between estimates of GCV and PCV for characters like days to 50 per cent flowering, plant height, pod width and pod weight indicating predominant genetic contribution and less environmental influence. Higher values of GCV and PCV were exhibited for seed yield per plant (25.52% and 39.40%), pods per plant (23.81% and 36.63%), racemes per plant (16.64% and 29.63%), pods per raceme (16.05% and 26.27%), pod weight (20.35% and 25.38%) and pod width (22.78% and 25.06%). Moderate to low GCV and PCV estimates were found for plant height (13.19% and 18.34%), seeds per pod (5.26% and 12.14%), pod length (1.82% and 11.84%), days to 50% flowering (2.24% and 4.03%) and days to maturity (0.42% and 3.94%) which indicated the influence of the environment on these traits and limited scope for improvement by selection. Results were in line with Hadavani *et*

*al.* (2018), Jyothireddy *et al.* (2018a) and Noorjahan *et al.* (2019).

High heritability coupled with high genetic advance expressed as percentage of mean was observed for pod width (82.60%, 42.64%) and pod weight (64.30%, 33.61%). These characters may have contributed to preponderance of additive gene action and selection pressure could profitably be applied on these characters for improving the seed yield per plant Moderate heritability with high to moderate genetic advance as per cent of mean was observed for pods per raceme (37.30%, 20.20%), pods per plant (42.30%, 31.89%), seed yield per plant (41.90%, 34.04%), plant height (51.70%, 19.54%) and racemes per plant (31.50%, 19.24%). Selection would be effective for improvement of these traits. Similar results were observed by Gupta *et al.* (2010), Parmar *et al.* (2013) and Sharma *et al.* (2014).

Genotypic correlation coefficients for 11 pair of characters are mentioned in Table 3. Seed yield per plant had positive and highly significant correlation with days to maturity (0.850), plant height (0.526), racemes per plant (0.355), pods per raceme (0.460), pods per plant (0.434), pod width (0.552), pod weight (0.698) and seeds per pod (0.207). Similar results of association of these traits with seed yield per plant were reported by Pawar and Prajapati (2013) for days to maturity, plant height, pods per



plant, racemes per plant, pod length; Kamble *et al.* (2015) for days to flowering; Salim *et al.* (2013) for seeds per pod.

Path analysis revealed that pod weight (1.218) and pods per plant (0.770) exhibited positive direct effect of higher magnitude on seed yield per plant. This result is in agreement with the findings reported by Chattopadhyay and Dutta (2010), Magalingam *et al.* (2013), Chaitanya *et al.* (2014) and Jyothireddy *et al.* (2018b).

Residual effect indicates unaccountable variation in path analysis. In this study, residual effect of 0.207 was observed suggesting that traits under consideration could account for 80 % of total variation. Other traits may be included in future studies to increase percent accountable variation in the experiment.

## CONCLUSION

Analysis of variance revealed highly significant variation among all the yield attributing traits under study. Higher amount of variability observed within cross B, cross C and cross D as well for majority of traits. Higher values of GCV and PCV were exhibited for seed yield per plant, pods per plant, racemes per plant, pods per raceme, pod weight and pod width. High heritability coupled with high genetic advance expressed as percentage of mean was observed for pod width and pod weight. Also, pod weight and pods per plant exhibited highly significant and positive association and higher magnitude of positive direct effect on seed yield per plant and hence, these may be considered as most important yield contributing characters and due emphasis should be placed on these components while applying selection for high seed yield in Indian bean.

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# Feasibility of Vacuum based Cooling System for on Farm Cooling of Milk

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## ABSTRACT

India ranked 2<sup>nd</sup> in term of population and 1<sup>st</sup> in milk production but still the value addition of milk is about 23%. However, increasing incidences of malnutrition throughout the world, it become necessary that every grain of food and each drop of milk must be saved and delivered to the consumer in a scientific manner. Though, milk is considered as complete food, but its perishable nature resulted in its easy spoilage, if not stored at proper temperature specially in tropical country like India. The microbes easily grow in the ambient temperature, so the cooling of milk immediately after milking is necessary to suppress the growth of microbe. Most of the current cooling systems are based on the refrigerants like R134a (HFC refrigerant) having high coefficient of performance but, these refrigerant have disadvantage of emission of harmful gases like CFCs which results in global warming and ozone depletion. A double cavity insulated system was designed with vacuum assembly. Vacuum based cooling system for on farm cooling of milk which helps to cold supply chain of milk and help to prevent the milk spoilage. In this cooling system water used as refrigerant so it is eco-friendly. With the designed system, the milk temperature drops from 37 °C to 12.5 °C within one hour so it may possible to prevent the milk spoilage up to 8 hours.

## HIGHLIGHTS

- In this cooling system water is used as refrigerant for milk cooling
- In this vacuum based cooling system milk temperature decreased from 37 °C to nearly 12.5 °C with in 3000 second.
- This cooling system is open cooling system, so it removes the necessities of accessories like compressor, expansion valve, receiver etc. which are required for most of the current cooling system.

**Keywords:** Malnutrition, Milking, Global warming, Vacuum, Milk spoilage

Milk and milk products get spoiled due to the growth of fermentative bacteria during the storage at higher temperature. For maintaining milk quality and to reduce losses cooling down the temperature of milk is the important factor. Reduced temperature helps to reduce the biochemical, physiological and microbial activities, which are responsible for quality deterioration. If milk stored at temperature 30 °C and above, at and below 20 °C, at and below 10 °C and below 4 °C the milk spoilage hours can be increased up to four, eight, 16 and 24 hours

respectively (Torres- Toledo *et al.* 2018). According to the climatic condition of India which provide nearly ambient temperature for the growth of microorganism, cooling system with insulation is required for milk cooling to enhance its shelf-life.

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Soylemez *et al.* (2018) reported that the most of current refrigeration system are based on refrigerants like R134a (an HFC refrigerant) etc., these refrigerants having high COP (coefficient of performance) but have also disadvantage of emission of harmful gases like CFCs which cause to increase global warming and depletion of ozone layer. So, there are need of green and eco-friendly technologies for cooling purpose.

The cooling system either vapour compression refrigeration system or vapour absorption refrigeration system use the same refrigerant in cyclic process, so these cooling system require components like evaporator, compressor, condenser, expansion valve, pump, generator etc. to make the process cyclic. In this open refrigeration system once the vapour of refrigerant (water) formed in evaporator are either released directly to the environment or absorbed but not reused for producing cooling effect so, this open refrigeration system not require much component as require in vapour compression or vapour absorption refrigeration system.

Double cavity insulated system was used for milk cooling by open refrigeration system. The outer cavity of can work's as evaporator section in which evaporation of refrigerant (water) occurs which extract heat from inner cavity fluid by changing phase from liquid to vapour. Vacuum pump was connected to outer cavity to create vacuum in outer cavity so the refrigerant (water) can easily evaporate at lower temperature and produce cooling effect in available cooling system. The study was aimed at exploring the feasibility of milk cooling using water as refrigerant and eliminating the compressors and condensers of conventional refrigeration system.

## MATERIALS AND METHODS

### Double Cavity system

Double cavity system available in Department of Dairy Engineering, College of Dairy Science and Technology, GADVASU, having inner cavity made up SS - 304 with capacity 550 ml and outer cavity made up SS - 304 with capacity 330 ml and having two connections in outer cavity for vacuum pump, pressure gauge, refrigerant (water) inlet/outlet. Insulation was done with the help of PUF as insulating material. The accessories used, vacuum pump, vacuum gauge, one-way valve etc.

Table 1 shows the dimensions of double cavity can prototype. Fig. 1 shows double cavity system.

**Table 1:** Dimension of double cavity system

	Height (mm)	Diameter (mm)
Outer cavity	470	52
Inner cavity	560	40

### Vacuum Pump

High vacuum pump (flowtech) Model-DC:300 was used. The table 2 shows the specifications of vacuum pump.

**Table 2:** Specifications of vacuum pump

Specifications	Flow tech
Frequency (Hz)	50
Motor power (W)	1 HP/220v
Motor speed (rpm)	1440
Oil capacity (mL)	750

### Methodology Applied

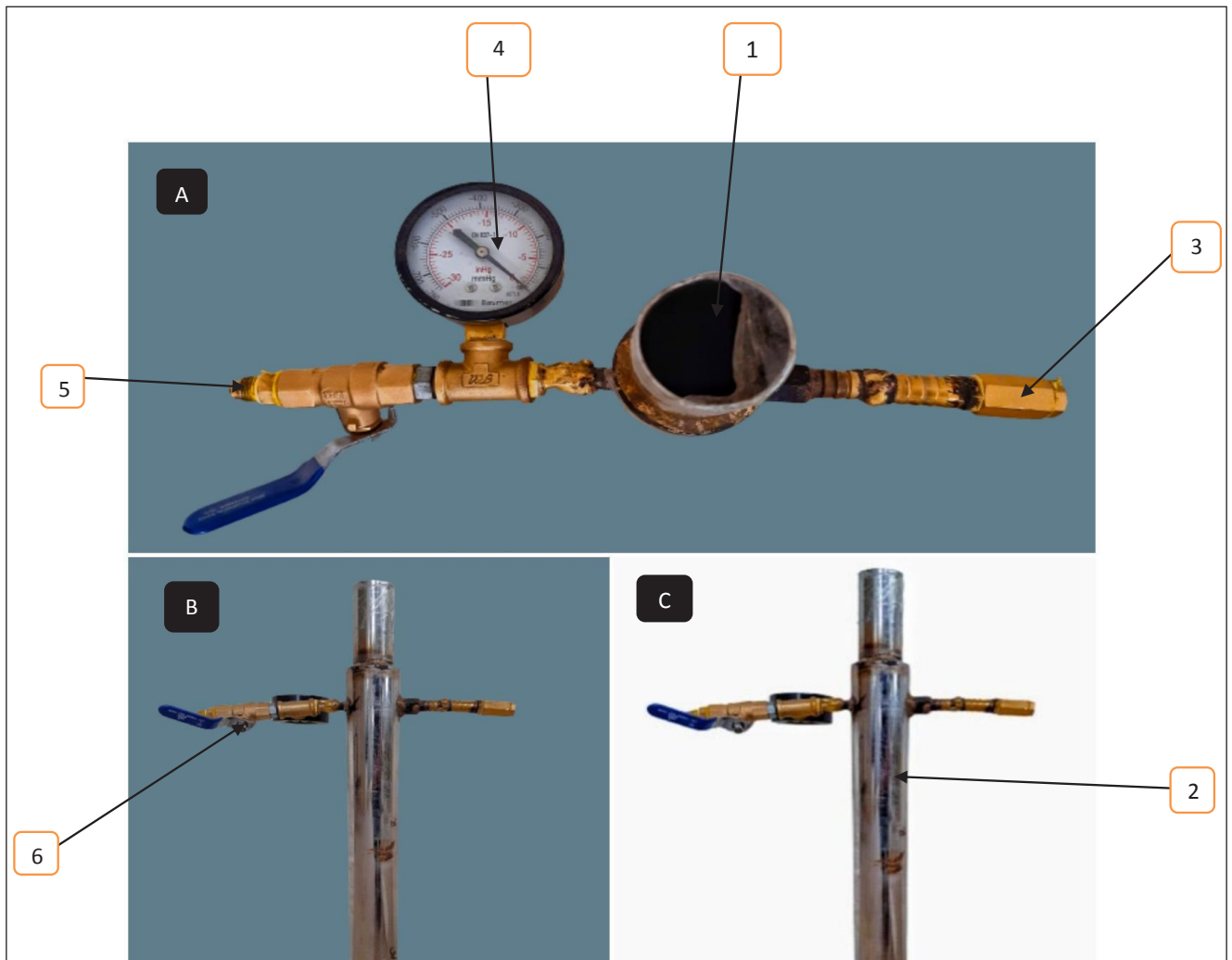
Vacuum pressure in outer cavity was optimized to achieve the desired cooling effect and cooling rate during pressure optimization quantity of water was 200 and 500 ml in outer and inner cavity was taken and orientation of double cavity system was vertical. Various vacuum level 620,660,690 and 700 mmHg were applied. The product to refrigerant ratio was optimized to ascertain the requirement of refrigerant (water), 500 ml water in inner cavity and 50 ml, 100 ml, and 200 ml were tried in outer cavity during product to refrigerant ratio optimization 700mmHg vacuum was maintained in outer cavity and orientation of double cavity system was vertical. Two orientation vertical and horizontal of double cavity insulated system was applied by taking 200ml water and 700 mmHg vacuum in outer cavity. Intermittent operation on vacuum pump by partial run was tried to minimize the energy consumption (300 sec turn on and 600 sec turned off). Finally, the cooling effect of optimized parameter vacuum in outer cavity, quantity of refrigerant in outer cavity and orientation of double cavity system on milk.

## RESULTS AND DISCUSSION

### 1. Optimization of pressure in outer cavity for cooling effect in inner cavity

Various vacuum level was maintained in outer





1- Inner cavity, 2- Outer cavity, 3- Refrigerant Inlet/outlet, 4- Vacuum gauge, 5- Vacuum pump connection point, 6- One-way valve

**Fig. 1:** Double cavity system (A) Top view, (B&C) Side views

cavity and minimum reached temperature was note down, the methodology is already discussed. The results are showing in table 3 at 620 mmHg vacuum minimum reached temperature – 33 °C, at 660 mmHg vacuum minimum temperature – 29 °C, at 690 mmHg vacuum minimum temperature – 22.8 °C and at 700 mmHg vacuum minimum temperature achieved was 14.3 °C, this shows that as the vacuum level in outer cavity increasing the minimum temperature achieved was decreasing.

These shows that the increasing vacuum/decreasing the atmospheric pressure above the water surface the boiling point of water is decrease, the boiling point of water at that achieved vacuum level in outer cavity is still far from the water temperature in outer cavity, but still due to the vacuum the rate of evaporation of water in outer cavity was high,

which help to produce cooling effect. The boiling points are as at 610.6 mmHg vacuum – 60 °C, at 667.5 mmHg vacuum – 50 °C, and at 704.9 mmHg vacuum boiling point of water is 40 °C (standard boiling point of water at different vacuum level).

As shown in Fig. 2 the vacuum level increasing in outer cavity the decrease in temperature in inner cavity is increasing, which shows at higher vacuum level higher the rate of evaporation of water and higher the rate of heat extraction from available water and reduce the temperature. Due to this a high temperature difference between the outer cavity fluid (refrigerant) and inner cavity fluid (product), so initially the rate of heat transfer was higher but it starts to decrease due to the decrease in temperature gradient between outer and inner fluid. As shown in Fig 2. at 690 mmHg vacuum the

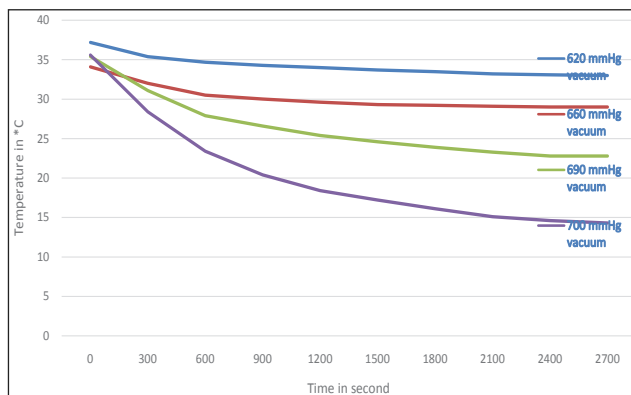
**Table 3:** Temperature profile of inner cavity fluid at various vacuum level

Duration (second)	Water in inner cavity (ml)	Temperature (+/-1)(°C) profile of inner cavity fluid at vacuum level 620+/- 5 (mmHg)	Temperature (+/-1) (°C) profile of inner cavity fluid at vacuum level 660+/- 5 (mmHg)	Temperature (+/-1) (°C) profile of inner cavity fluid at vacuum level 690+/- 5 (mmHg)	Temperature (+/-1) (°C) profile of inner cavity fluid at vacuum level 700+/- 5 (mmHg)
0	500	37.2	36.4	35.4	35.6
300	500	35.4	34.1	31.1	28.4
600	500	34.7	32.0	27.9	23.4
900	500	34.3	30.5	26.6	20.4
1200	500	34.0	30.0	25.4	18.4
1500	500	33.7	29.6	24.6	17.2
1800	500	33.5	29.3	23.9	16.1
2100	500	33.2	29.2	23.3	15.1
2400	500	33.1	29.1	22.8	14.6
2700	500	33.0	29.0	22.8	14.3

**Table 4:** Temperature profile of inner cavity product with various water in outer cavity

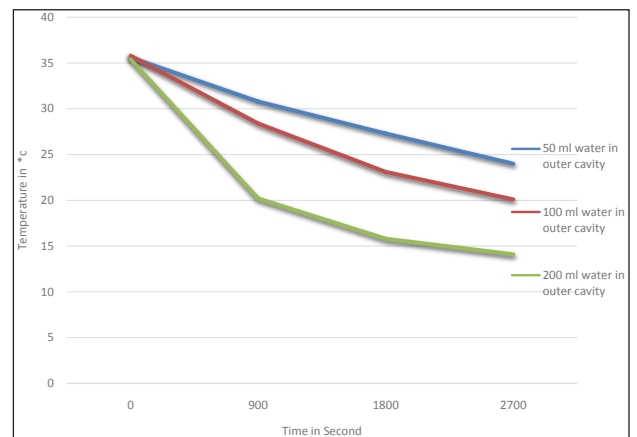
Duration (sec)	Quantity of water in inner cavity (ml)	Vacuum in outer cavity (+/- 5) (mmHg)	Temperature (+/-1) (°C) profile of inner cavity fluid with 50 ml water in outer cavity.	Temperature (+/-1) (°C) profile of inner cavity fluid with 100 ml water in outer cavity.	Temperature (+/-1)(°C) profile of inner cavity fluid with 200 ml water in outer cavity.
0	500	700	35.6	35.8	35.4
900	500	700	30.8	28.4	20.2
1800	500	700	27.3	23.1	15.8
2700	500	700	24.0	20.1	14.1

minimum temperature 22.8 °C was achieved after 2700 second on the other hand 20.4 °C temperature was achieved with in 900 sec at 700 mmHg vacuum.



**Fig. 2:** Temperature profile of inner cavity fluid at various vacuum level

The Fig. 3 is showing that when quantity of refrigerant/water increasing in outer cavity, cooling effect is also increasing in inner cavity.



**Fig. 3:** Temperature profile of inner cavity product with different quantity of refrigerant in outer cavity

## 2. Optimization of quantity of refrigerant (water) in outer cavity for cooling effect in inner cavity product

The quantity of water in inner cavity was taken 500ml in all experiments.

This was due to the increase in quantity of water in outer cavity there was increase in area of contact between the outer and inner cavity fluid and according to Fourier law of heat conduction the rate of heat transfer is directly proportional to the

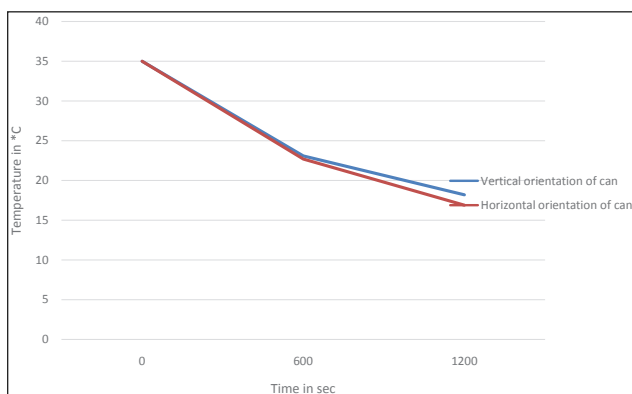
area, so the rate of heat transfer from inner cavity fluid to outer cavity fluid was more when 200 ml water in outer cavity as compare to 50 ml, 100 ml water in outer cavity.

When quantity of water was 50 ml in outer cavity, it covers very less surface area of inner cavity or the contact area between outer and inner cavity fluid was very less. The heat transfer phenomenon in covered area was as from hot fluid to metal (can) by convection then in metal over thickness by conduction then again from metal to fluid by convection, but heat transfer phenomenon is completely different in uncovered area, in this heat transferred from hot fluid to metal (can) by convection then conduction of heat in metal over length as hollow cylinder then again from metal (can) to cold fluid by convection. This is the reason when the quantity of water increasing from 50 ml to 200 ml then the rate of heat transfer increasing.

By this experiment the larger size pool 200 ml was optimized to maximize the area of contact between outer and inner cavity fluid.

### 3. Optimization of surface area for evaporation of refrigerant (water) in outer cavity under vacuum

Evaporation of refrigerant (water) in outer cavity under vacuum is producing cooling effect, evaporation is a surface phenomenon and it is directly proportional to the available surface area. The increase in surface area for evaporation will help to increase the rate of evaporation which directly help to cooling rate.



**Fig. 4:** Temperature profile of inner cavity fluid with vertical and horizontal orientation of double cavity system

The Fig. 4 showing the rate of heat transfer is faster in horizontal orientation, this was due to

the more surface area available in outer cavity for evaporation.

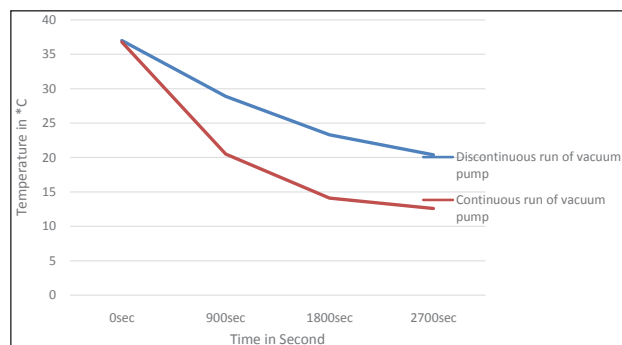
On the basis of this experiment the horizontal orientation of double cavity system was selected. The change in orientation of system was the only option to optimize the area of evaporation in outer cavity for the available double cavity experimental setup.

### 4. Effect of intermittent operation of vacuum pump, continuous and partial run of vacuum pump

Quantity of water 500 ml and 200 ml in inner and outer cavity, the optimized vacuum 700 mmHg was created in outer cavity and the orientation of can was horizontal.

The table 7 showing that the temperature recording of inner cavity fluid with continuous and discontinuous run of vacuum pump. In discontinuous operation vacuum pump run for 300 sec and then turned off for next 600 sec. When vacuum pump run continuously the inner cavity fluid temperature decreases from 36.8 °C to 12.6 °C and it decreases only 37 °C to 20.4 °C for discontinuous run of vacuum pump.

Fig. 5 showing that the cooling rate of continuous running is high as compare to the discontinuous running vacuum pump. In continuous operation vacuum pump run for 2700 sec and in discontinuous operation vacuum pump run for 900 sec only. In discontinuous operation after 2700 sec final temperature was recorded 20.4 °C, that temperature was achieved just after 900 sec running of vacuum pump in continuous operation.



**Fig. 5:** Temperature profile of inner cavity fluid with intermittent operation on vacuum pump

Cooling effect is due to the evaporation of refrigerant (water) at low pressure in outer cavity, liquid

**Table 5:** Temperature profile of inner cavity fluid in vertical orientation of can

Duration (sec)	Quantity of water in inner cavity (ml)	Quantity of water in outer cavity (ml)	Vacuum in outer cavity (+/- 5) (mmHg)	Temperature (+/-1) in inner cavity (°C)	Cumulative decrease in temperature in inner cavity (°C)
0	500	200	700	35	0
600	500	—	700	23.1	11.9
1200	500	—	700	18.0	5.1

**Table 6:** Temperature profile of inner cavity fluid in horizontal orientation of can

Duration (sec)	Quantity of water in inner cavity (ml)	Quantity of water in outer cavity (ml)	Vacuum in outer cavity (+/- 5) (mmHg)	Temperature (+/-1) in inner cavity (°C)	Cumulative decrease in temperature in inner cavity (°C)
0	500	200	700	35	0
600	500	—	700	22.7	12.3
1200	500	—	700	17.1	5.6

**Table 7:** Temperature profile of inner cavity fluid with intermittent operation of vacuum pump

Duration (sec)	Quantity of Water in inner cavity (ml)	Quantity of Water in outer cavity (ml)	Vacuum in outer cavity (+/- 5) (mmHg)	1 <sup>st</sup> – Temperature profile of inner cavity fluid with discontinue run of vacuum pump (300sec run then turned off for next 600sec)	2 <sup>nd</sup> – Temperature profile of inner cavity fluid with continue run of vacuum pump
0	500	200	700	37	36.8
900	500	—	700	28.9	20.5
1800	500	—	700	23.3	14.1
2700	500	—	700	20.4	12.6

**Table 8:** Temperature profile of inner cavity milk

Duration (Min.)	Quantity of water in outer cavity (ml)	Quantity of milk in inner cavity (ml)	Vacuum in outer cavity (+/- 5) (mmHg)	Average decrease in temperature(+/-1) of milk (°C)	Cumulative decrease in temperature of milk (°C)
0	200	500	700	35.7	0
300	—	500	700	28.1	7.6
600	—	500	700	23.4	4.7
900	—	500	700	20.4	3
1200	—	500	700	17.6	2.8
1500	—	500	700	15.6	2
1800	—	500	700	14.2	1.4
2100	—	500	700	13.1	1.1
2400	—	500	700	12.3	0.8
2700	—	500	700	12.2	0.1
3000	150	500	700	12.1	0.1

converts to gas (vapour) by taking heat. When vacuum pump runs then these vapours are removed from outer cavity and produce cooling effect but in discontinuous operation when vacuum pump turned off then the adiabatic process occurred in outer cavity no heat exchange. When vacuum pump turned off then vapour still forming in outer cavity but these vapours condensing at the same time in outer cavity, due to this vapour comes in

equilibrium state and cannot be able to produce cooling effect.

This experiment was designed to save energy by reducing the running time of vacuum pump but continuous removal of vapour from outer cavity is necessary to produce cooling effect. So, either continuous run of vacuum pump or continuous removal of vapour from outer cavity is necessary to produce cooling effect.

### 5. The cooling effect of optimized parameter vacuum in outer cavity, quantity of refrigerant in outer cavity and orientation of double cavity insulated system on milk

500 ml milk was taken in inner cavity and 200 ml water filled in outer cavity. The orientation of can was horizontal, vacuum 700 mmHg maintained in outer cavity and vacuum pump runs continuously. Table 8 showing the temperature profile of inner cavity milk. The cumulative decrease in temperature of milk was nearly 7.6 °C during 1<sup>st</sup> 300 sec and cumulative decrease was 0.1 °C during last 300 sec which shows as time runs the cooling rate is decreases due to the decrease in temperature gradient of inner and outer cavity fluid. Initially the cooling rate was high due to the high rate of evaporation which depends on temperature of fluid.

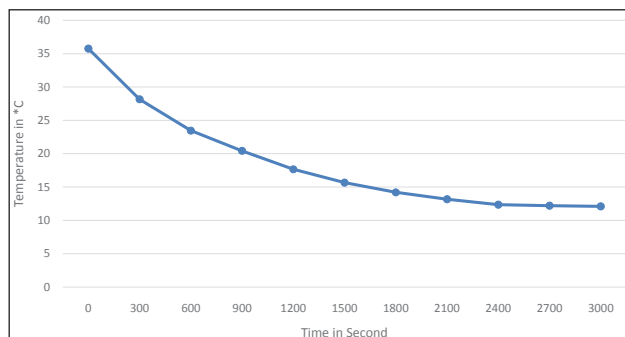


Fig. 6: Temperature profile of Milk

### CONCLUSION

This study for the feasibility of vacuum based cooling system for on farm cooling of milk showing that the temperature of milk cools down from nearly 36 °C to 12.5 °C with in 3000 sec (50 minute) which may help to preserve the quality of raw milk up to 8 hours. This system is using water as refrigerant so eco-friendly cooling system. This cooling system is open cooling system because refrigerant used only once (not in cycle) so it also removes the necessities of accessories like compressor, expansion valve, receiver etc.

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# A Study on Marketing Channels and Marketing Efficiency of Capsicum in Mid-Hills of Himachal Pradesh

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## ABSTRACT

The present study is to identify different marketing channels and marketing efficiency of capsicum in Mid-hills of Himachal Pradesh. Himachal Pradesh provides opportunity for production of different vegetables for sustainable income to farmers. Marketing of horticultural crops is complex especially because of perishability, seasonality and bulkiness. Two blocks (Balh and Kangra) were selected purposively from Mid-hill zone of Himachal Pradesh. A sample of 100 farmer's cultivating different vegetables and 30 market intermediaries (15 retailers; 10 wholesalers; 5 Local traders) has been selected on multistage random sampling technique. Four major marketing channels identified in the study were channel-A: (Producer-Consumer), channel-B: (producer-wholesaler-retailer-consumer), channel-C: (Producer-Local Trader-Wholesaler-Retailer-Consumer) and channel-D: (Producer-Retailer-Consumer). The price spread was low in channel-A as the produce was sold to the consumer directly by the producer. Comparison between different channels revealed the highest share in consumer's rupee in Channel A (Producer-consumer) and marketing efficiency has been also highest in channel A. The most important marketing channel is use in the study area is that of Channel-B (Producer-Wholesalers-Retailer-Consumer). As a result, there is a need for providing regulated and subsidized transportation facilities to the farmers in order to reduce marketing costs and thereby increase their share of the consumer's rupee.

## HIGHLIGHTS

- ① The Purpose of the study was to study the compare the channel efficiency of the capsicum growers. Keeping in view high capsicum production and marketing in the study area it was imperative to study price realization at farm level.
- ① Channel-A was found most efficient channel but quantity of produce sold through this channel was lower than other channels. From the remaining three channels, Channel-B was found to be the most preferred channel by the vegetable growers.
- ① It highlights the need to enhance efficiency of the market channel-B (Producer-W-R-C) and channel-C (Producer-Local Trader/Commission agent -W-R-C) through competition by organized marketing chains and modernizing the vegetable market system in the district, which is largely traditional and lacks modern facilities such as efficient transportation of produce and grading & standardization facilities.

**Keywords:** Marketing channels, price spread, marketing efficiency and capsicum

Agriculture, forestry, and fishery had a gross value added of ₹ 19.48 lac crore (US\$ 276.37 billion) in fiscal year 2020. In fiscal year (FY) 2020, agricultural and allied industries accounted for 17.80 per cent of India's gross value added (GVA) at current prices. In 2021, consumer spending in India could

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rise by up to 6.60 per cent. India's share of global agricultural exports increased to 2.10 per cent in 2019 from 1.71 per cent in 2010 (Ministry of Commerce 2021). Vegetables play a major role in Indian economy by producing higher returns per unit area and time, besides they provide nutritional and economic security. The demand for vegetables is continuously increasing at a faster rate due to increasing population pressure, increasing awareness of nutritional value of vegetables and as a result of the increasing per capita income over time. Vegetables have higher productivity, shorter maturity cycle, high value and provide higher income leading to improved livelihood. The vegetable crops also offer better crop diversification and intensification. The cultivation of better quality vegetable crops have a much higher export potential not only to neighbouring states in the country but have recently found way to the African/South Eastern countries as compared to field crops and thus help to generate a valuable foreign exchange (Arya 2001). In India, vegetables are grown in an area of 11,065 thousand hectares with a production of 1, 99,882 thousand metric tonnes (NHB, 2021). West Bengal is the largest vegetable producer in the country (15.35 %) followed by Uttar Pradesh (14.46 %), Madhya Pradesh (10.18%), Bihar (8.91 %), Maharashtra (7.84 %), Gujarat (6.74 %), Odisha (4.55 %), Karnataka (3.69 %) and Himachal Pradesh (0.94%) (NHB, 2021). Vegetable production plays important role in poverty reduction by generating jobs, improving the feeding behaviour of the people and creating new opportunities for poor farmers. Since the vegetable products are bulky, perishable and has continuous demand in the market, its production and marketing allows high productivity employment. Increased horticultural production and marketing thus contribute to commercialization of the rural economy and the creation of numerous off-farm jobs (Weinberger and Lumpkin 2005).

Himachal Pradesh has diverse agro-climatic conditions that support the cultivation of nearly all kinds of vegetables, both temperate and subtropical in nature (Chauhan 2004; Chauhan and Mehta 2002). The state economy is mainly based on agriculture as about 69 per cent of workers depend on agriculture. Moreover, the varied agro-ecological conditions of the state have an advantage over the plains in growing a number of vegetable crops due to their

varying growing seasons. This has made vegetable production highly profitable, particularly in mid and high hills of the state (Sharma 2007). The area under vegetables in the state is 9199 thousand hectares with a production of 1,875.24 thousand metric tonnes and a productivity of 20.38 MT/ha. Out of which the total area under capsicum is 2.85 thousand hectares and total production is 48.86 thousand metric tonnes (Directorate of Economics and Statistics 2021).

Marketing of vegetable crops is quite complex and risky due to the perishable nature of the produce, seasonal production and bulkiness. Farmers that can market their produce in the right form at right time and place for the right price emerge successful, while the rest give up their fair share to middlemen or traders. Being perishable in nature, vegetable crops require a quick and efficient marketing system. An efficient marketing system is important for economic development because it stimulates production, avoids unnecessary fluctuations in output and prices, reduces production costs and an unfair share of consumer prices. The present study was undertaken in Mid hills of Himachal Pradesh to analyze the marketing channels and marketing efficiency of capsicum.

## MATERIAL AND METHODS

### Sampling design

Multistage random sampling was adopted in the selection of blocks, Gram Panchayat and the ultimate sample of the respondents who were involved in marketing of vegetables.

### Study area

At first stage, Two blocks (Balh and Kangra) were selected purposively from Mid-hill zone of Himachal Pradesh. At the second stage, a list of Gram Panchayats from each selected blocks was prepared and out of which 5 Gram Panchayats were selected from each selected block. At the third stage, a list of vegetable growers of the selected Gram Panchayat was prepared and out of which 10 farmers from each selected Gram Panchayat were selected randomly for collection of the primary data. Thus, sample of 100 vegetable growers were selected for the present study.



## Selection of market and market intermediaries

Two markets, namely Balh and Kangra were purposively selected to collect the information related to markets and marketing. A sample of 5 local traders/commission agents, 10 wholesalers, 15 retailers and 30 consumers were selected randomly for gathering the data of vegetable marketing in Dhanotu block (Mandi district) and Kangra block (Kangra district). Both primary as well as secondary data were collected during the survey investigation. The primary data were collected using pre-tested schedules through personal interview method from the selected households and Traders/Commission agents in the study area and markets. Secondary data were collected from the Department of Agriculture, Agricultural Produce Market Committee (APMC), traders/commission agents and wholesalers associations of the selected market.

## Analytical framework

### Total marketing cost

The total cost incurred on marketing by the vegetable growers and various intermediaries involved in the sale and purchase of the commodity till the commodity reaches to the ultimate consumer, may be computed as follows:

$$C = C_F + C_{m1} + C_{m2} + C_{m3} + \dots + C_{mn}$$

Where,

$C$  = Total cost of vegetable marketing,

$C_F$  = Cost paid by the producer in the marketing of his produce

$C_{mi}$  = Marketing costs incurred by  $i^{\text{th}}$  middleman

### Marketing Margin

Marketing margin of middlemen calculated as the difference between the total payments (marketing cost + purchase price) and receipts (sale price) of the middlemen and calculated as follows.

$$A_{mi} = P_{Ri} - (P_{pi} + C_{mi})$$

Where,

$A_{mi}$  = Absolute margin of middlemen

$P_{Ri}$  = Total value of receipts per unit (sale price)

$P_{pi}$  = Purchase value of goods per unit

$C_{mi}$  = Cost incurred on marketing per unit

### Net price received by producer

The net price received by the producer has been calculated by deducting the marketing costs borne by the producers from the original price paid to the producers by the commission agent/ wholesaler and is calculated as given below.

$$P_F = P_S - P_C$$

Where,

$P_F$  = Net price received by the producer.

$P_S$  = Producer's selling price.

$P_C$  = Marketing cost incurred by the producers.

### Producer's share in consumer's rupee

It is the price received by the producer expressed as a percentage of the retail price (i.e., price paid by the consumer). It has been worked out as under,

$$PS = \frac{FP}{RP} \times 100$$

Where,

$PS$  = Producer's share in consumer's rupee

$FP$  = Price received by the farmer per unit of output

$RP$  = Retail price per unit of output

### Price spread

The difference between the price paid by the consumer and price received by the producers was the total marketing margin or price spread. Generally, the economic efficiency of marketing system is measured in terms of price spread. Smaller the price spread; greater is the efficiency of the marketing system. Price spread was calculated as per Kashyap and Guleria (2015):

$$\text{Gross Marketing margin (GMM)} = \text{Selling price (SP)} - \text{Purchase price (PP)}$$

### Marketing efficiency of the marketing channels

In case of marketing channels, the marketing efficiency is concerned with the movement of goods from producer to consumer at the lowest possible



cost consistent with the provision of services desired by the consumers. The marketing efficiency of various channels in the study area has been computed by using Acharya's method, (Acharya and Agarwal 2001, as under:

$$ME = \frac{RP}{MC + MM} - 1$$

Where,

ME = Marketing efficiency; RP = Retailer's price; MC = Total marketing costs; MM = Total marketing margins

## RESULTS AND DISCUSSION

Himachal Pradesh is located in the Northern region of India, surrounded by Jammu & Kashmir, Punjab, Haryana, Uttar Pradesh and China on the four sides. Geographically, it extends between 30°22'N and 33°12'N latitude and 75°47'E ' and 79°04'E longitude. The state is small hill state, with altitude varying from 300 m in Kangra and Una to nearly 7000 m in Central Himalayan range of Lahaul & Spiti. Himachal Pradesh agro-climatically has been divided into four zones keeping in view the altitude, rainfall, temperature, humidity and topography.

### Marketing channels

Marketing channels are routes through which agricultural products move from producers to consumers. The length of the channel varies from commodity to commodity, depending on the quantity to be moved, the type of consumer demand and degree of regional specialization in production. Due to the existence of various agencies working between producer and consumer, there are different marketing channels that exist for the same commodity. The agencies involved in the marketing of various vegetables in the study area are local traders, wholesalers and retailers. Four marketing channels were indentified in the study area for marketing of capsicum.

Marketing channels	Marketing intermediaries
Channel-A	Producer → Consumer
Channel-B	Producer → Wholesaler → Retailer → Consumer
Channel-C	Producer → Local Trader → Wholesaler → Retailer → Consumer
Channel-D	Producer → Retailer → Consumer

**Market intermediaries:** Three types of production system were observed in the study area viz. subsistence production, small scale commercial production and the large scale commercial production. The produce from the first category of farmers generally does not enter the market or enters in a very small quantity especially in the local market. Small and large scale commercial farmers sell most of the produce to various market intermediaries. Due to the existence of various actors working between producer and consumer, there are different marketing channels for the same commodity. The actors involved in the marketing of capsicum in the study area are local traders, wholesalers and retailers. The four marketing channels were patronized for the marketing of capsicum in the study area (Singh *et al.* 2020).

**Local trader/commission agent:** Local Traders/ Commission agents are the key actors of the capsicum value chain who are involved in trading vegetables from production pocket to the wholesale markets. Their trading activities include: buying and assembling, repacking, sorting, selling to middlemen, transporting and selling to wholesale markets.

**Wholesalers:** Wholesalers are mainly involved in buying vegetables from collectors and producers in larger volume than any other actors and supplying them to retailers and consumers. Survey result indicates that wholesale markets are the main assembly centers for capsicum in their respective surrounding areas. They have better storage, transport and communication access than other Traders/Commission agents. In the study area, market yards located in Dhanotu, Chail Chowk, Kangra and Nagrota bagwan.

**Retailers:** Retailers are the last link between producers and consumers. They mostly buy from wholesalers and sell to consumers. Sometimes they could also directly buy from the producers. Consumers usually buy the product from retailers as they offer according to requirement and purchasing power of the buyers.

**Consumers:** Consumer is an individual who buys products or services for personal use and not for manufacture or resale. It is an end user in the value chain of capsicum.

### (a) Direct sale to consumer

The channel shows a direct relationship with the consumer. Table 1 analyzed the quantity of capsicum marketed through different channels. Farmers sold nearly 5.40 per cent of their produce directly to consumers. Channel-A was patronized on a limited scale as this took more time for the sale of capsicum in spite of its high efficiency.

**Table 1:** Average quantity of capsicum marketed through various marketing channels (Quintal/farm)

Marketing channels	Quantity Handled
Channel-A	0.43 (5.40)
Channel-B	4.00 (50.70)
Channel-C	2.34 (29.66)
Channel-D	1.12 (14.25)
<b>Total</b>	<b>7.89 (100)</b>

Figures in parenthesis are percentage to total.

### (b) Sale through wholesaler

Wholesaler was the most commonly used market functionary in Channel-B and C. It can be observed that Channel-B was found to be the most preferred channel by the vegetable growers since 50.70 per cent of capsicum was traded through this channel.

### (c) Sale through local trader

The second important channel followed by the vegetable growers was Producer-local trader-Wholesaler-Retailer-Consumer. Local traders collected the produce from the farm gate of the producer and further sold it to wholesalers or retailers. In the study, no local trader was found to be directly dealing with the consumer. In case of cauliflower, growers sold nearly 29.66 per cent of produce was marketed through this channel. This channel was most preferred by the high hills zone.

### (d) Sale through retailer

Retailer collected the produce from the local traders and wholesalers and further sold it to the consumer. The retailer appeared in three channels i.e., Channel-B, C and D. The retailer was the only market functionary apart from the producer who sold the produce directly to the consumer. In case (14.25 %) of Capsicum was traded through Channel-D.

### Price spread for Capsicum

Price spread is the difference on ultimate price paid by the consumer and the net price received by the producer for an equivalent quantity of farm product. It consists of marketing cost and margins of the intermediaries that determines the overall effectiveness of marketing system.

Table 2 showed that the price received by farmers was highest in channel-A (₹ 3303.00/qtl) followed by channel-D (₹ 3159.00/qtl), channel-B (₹ 2852.00/qtl) and lowest in channel-C (₹ 2603.00/qtl). Per quintal total marketing costs varies between ₹ 47.00 to ₹ 972.09/ qtl among different channel. Channel-A (*Producer-Consumer*) was found to be the most efficient channel according to Acharya's method as the producer's share in consumer's rupee was also maximum in this channel. Similar finding were observed by Sashimatsung *et al.* (2013); Singh *et al.* (2018). It is observed that maximum quantity of Capsicum was marketed through channel-B. The gross marketing margins were maximum in channel-C (₹ 1533.19/qtl) and minimum in channel-D (₹ 529.60/qtl). A similar finding was reported by Sashimatsung *et al.* (2013). The marketing efficiency was highest in channel-A (70.28) followed by channel-D (5.96), channel-B

**Table 2:** Total marketing costs, margins and marketing efficiency for capsicum in different channels

Sl. No.	Particulars	Channel-A	Channel-B	Channel-C	Channel-D
1	Price received by farmers (₹/Qtl)	3303.00 (98.60)	2852.00 (68.98)	2603.00 (62.93)	3159.00 (85.64)
2	Total marketing costs (₹/Qtl)	47.00 (1.40)	809.60 (19.58)	972.09 (23.50)	337.30 (9.14)
3	Total net marketing margins (₹/Qtl)	—	472.90 (11.44)	561.09 (13.57)	192.30 (5.21)
4	Gross marketing margins (₹/Qtl)	—	1282.50 (31.02)	1533.19 (37.07)	529.60 (14.36)
5	Consumer's price (₹/Qtl)	3350.00 (100)	4134.50 (100)	4136.19 (100)	3688.60 (100)
6	Producer's share in consumer's rupee (%)	98.60	68.98	62.93	85.64
7	Marketing efficiency	70.28	2.22	1.70	5.96



(2.22) and least in channel-C (1.70) when calculated by Acharya's method.

Marketing costs and margins were high in channel-C due to a large number of market functionaries involved in the process of marketing of capsicum. Therefore, producer's share was less in comparison to the other channels. After the channel-A (*Producer-Consumer*), next profitable channel of producers for sale of Capsicum was through the Channel-D (*Producer-Retailer-Consumer*). The perusal of Table 2 showed that marketing efficiency for Capsicum in Channel-D (*Producer-Retailer-Consumer*) was higher than Channel-B and Channel-C. Channel-A (*Producer-Consumer*) was found to be most efficient channel as the producer's share in consumer's rupee was maximum in this channel. But, this channel is not preferred because the volume transacted was very less (5.40 %) than in other channels. From remaining three channels, Channel-B was identified to be the most preferred channel by vegetable farmers in the study area.

## CONCLUSION

From the study it was concluded that capsicum growers needed to diversify their market portfolio to realize better prices. It highlights the need to enhance efficiency of the market channel-B (*Producer-W-R-C*) and channel-C (*Producer-Local Trader/Commission agent -W-R-C*) through competition by organized marketing chains and modernizing the vegetable market system in the district, which is largely traditional and lacks modern facilities such as efficient transportation of produce and grading & standardization facilities. Channel-A (*Producer-consumer*) producers sold their produce directly to the consumers in the local market or on road side market. This channel was highly profitable for the farmer to undertake the marketing operations himself in order to realize higher margins for the produce. The producers share in consumers' rupee and marketing efficiency was highest in case of channel-A. Higher share of producer's in consumer's rupee was mainly due to the absence of market intermediaries. Channel-A was found most efficient channel but quantity of produce sold through this channel was lower than other channels. From the remaining three channels, Channel-B was found to be the most preferred channel by the vegetable growers. According to the

present study, the cost of loading and transportation is the highest of all marketing cost components. As a result, there is a need for providing regulated and subsidized transportation facilities to the farmers in order to reduce marketing costs and thereby increase their share of the consumer's rupee and increasing the number of market places, constructing rural godowns and cold stores for vegetables to strengthen the marketing infrastructure.

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# Evaluation of Biopesticides Against *Callosobruchus maculatus* Fabricius in Chickpea Under Stored Conditions

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## ABSTRACT

By realizing the significant losses caused by pulse beetles in storage and the importance of biopesticides, present investigation was carried out to evaluate eco-friendly biopesticidal approaches for management of *Callosobruchus maculatus* in chickpea. Four biopesticides viz., *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii* and *Bacillus thuringiensis* with wettable powder and oil formulations with wettable powder and oil formulations were evaluated as grain protectant against *Callosobruchus maculatus* Fabricius in stored chickpea. Among the various biopesticides tested, *B. bassiana* WP was found to be superior treatment with the highest half-life value (197.81), gross persistency value (7115). Significantly lower number of adults emerged in *B. bassiana* WP during six months of storage period. These affirmations can be considered for better storage of chickpea in large scale for long period of time and for sowing purpose.

## HIGHLIGHTS

- Among the various biopesticides tested, *B. bassiana* WP was found to be superior treatment with the highest half-life value (197.81), gross persistency value (7115). Significantly lower number of adults emerged in *B. bassiana* WP during six months of storage period.

**Keywords:** Biopesticides, Chickpea, *Callosobruchus maculatus*, Grain protectant, sustainable management

Chickpea is considered to be a healthy vegetarian food. It is a good source of dietary fibre, minerals (320 mg Ca, 275 mg P and 23.9 mg Fe) and vitamins (0.6 mg thiamine, 0.21 mg riboflavin and 1.9 mg niacin) (Duke 1981). With protein content of 29 % chickpea is a good source of protein (Hulse, 1991) and other contents include 59 % carbohydrate, 3 % fibre, 5 % oil and 4% ash. In India chickpea is grown over an area of 105.61 lakh ha with production of 112.29 lakh tonnes and productivity of 974 kg/ ha in India (Anon., 2018a).

In India, *Callosobruchus maculatus* and *C. chinensis*, commonly known as pulse beetles, are widely spread throughout the tropical and sub-tropical regions (Alam 1971). Infestations by the most prominent species are responsible to grain and losses are estimated to the tune of 20 to 60 per cent

(Tarver *et al.* 2007). *C. maculatus* is primary and the most destructive pest of stored pulses abundantly found in Gujarat. Infestation starts right from the field and carried to the storage. In stored condition maximum damage is caused in months of July to September (Butani *et al.* 2001). Pulse beetle causes not only quantitative but also qualitative losses like nutritive loss, germination loss and make the chickpea unfit for marketing as well as for human consumption. In India, pulse beetles breed freely from March to November and hibernates in the larval stage during winter.

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There is increasing evidence that Entomopathogenic Fungi (EPF) have potential for control of arthropod pest species in the storage environment. Entomopathogenic fungi are the diverse group of insect pathogens that contain a large number of genera and species. They are the only insect pathogens able to infect their host by adhesion to the surface and penetration through the cuticle (Mannino *et al.* 2019). By realizing the importance of biopesticides, present investigation was carried out to evaluate eco-friendly biopesticidal approaches for management of *C. maculatus* in chickpea.

## MATERIALS AND METHODS

The study was carried out during 2018 at Anand Agricultural University, Anand. About 300 adults were introduced in plastic jar (20 cm height and 14 cm diameter) containing 1 kg chickpea grains previously sterilized at 55 °C temperature for 4 hours in oven. The jar was covered tightly with muslin cloth affixed with rubber band to prevent the adults from escaping. Such jar containing chickpea grains and adults of *C. maculatus* was prepared and maintained in the laboratory for development and growth of the population. The damaged grains were replaced with undamaged and sterilized grains in each jar at monthly interval. The culture was maintained in the laboratory throughout the experimental period. The adults of *C. maculatus* obtained from the laboratory culture were used for further studies on biopesticidal management.

Four biopesticides *viz.*, *B. bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii* and *Bacillus thuringiensis* with wettable powder and oil formulations, all at concentration of  $2 \times 10^8$  cfu used. The chickpea variety GJG-3 was procured from Agriculture Research Station, AAU, Arnej and eight treatments of biopesticides were procured from AICRP on biological control, Unit 9, AAU, Anand. Each biopesticidal formulation was applied to 2.5 kg chickpea grain previously sterilized and smeared at a rate of 8 g/ kg seed or 8 ml/ kg seed (Shaik 2015). An untreated and sterilized bulk of 2.5 kg of chickpea grains was kept as control treatment. All the ten bulks (each of 2.5 kg) were stored in an air tight plastic bottle at room temperature and were utilized for the further experimentation. Treatments were evaluated for their efficacy as grain protectants against *C. maculatus* based on half-life, gross

persistence, population growth of pulse beetle and impact of biopesticides on germination of chickpea seeds during storage using Completely Randomized Design following the methodology described below.

## BASED ON HALF-LIFE

It was worked out by using following formula:

$$\text{Mortality half-life } (M_{1/2}) = \log 2 / K_1$$

Where,  $K_1$  = slope of the exponential regression of logarithms of number of adults died (after the addition of 1 to forestall the calculation of log 2) out of 20 against days passed (Kher 2006).

The mortality half-lives in days were worked out repetition-wise for each treatment. The data on half-lives were subjected to ANOVA.

## Gross persistency

The gross persistency of different biopesticides based on mortality of *C. maculatus* was worked out using following formula (Pawar and Yadav 1980).

Gross persistency =

$$\frac{\text{Sum of (percentage mortality} \times \text{period in days)}}{\text{Number of observations}}$$

For this purpose, data on corrected per cent mortality was worked out at 15 days interval and the gross persistency was calculated repetition-wise for each treatment. The data on gross persistency were subjected to ANOVA to check for significant difference among the various treatments.

## Based on population growth

The experiment was carried out in Completely Randomized Design (CRD) with 3 repetitions. For treatment, three samples of grains each of 50 g (one sample for one repetition) were filled in plastic tube (15 cm height and 7 cm diameter) individually. Five pairs of *C. maculatus* (3 days old) were released in each tube for egg laying and it was covered with two-fold muslin cloth kept in position by means of rubber band to prevent the adults from escaping. After 5 days, the adults were discarded. The observations on number of adults (live + dead) found in each tube were recorded after 3 and 5 months. The data on number of adults developed

after 3 and 5 months of storage were subjected to ANOVA after transforming them to square root.

## RESULTS AND DISCUSSION

The efficacy of biopesticides as grain protectants was evaluated based on half-life and gross persistency calculated repetition-wise for different biopesticides and was subjected to ANOVA. The data on half-life and gross persistency are presented in Table 1.

**Table 1:** Effect of biopesticides as grain protectant on *Callosobruchus maculatus* based on half-life and gross persistency during six months of storage in chickpea

Treatments	Formulation	Half life (Days)	Gross Persistency
<i>Beauveria bassiana</i>	WP	197.81a	7115a
<i>B. bassiana</i>	Oil	151.58b	6595b
<i>Metarhizium anisopliae</i>	WP	145.55b	5545c
<i>M. anisopliae</i>	Oil	123.30bc	5380c
<i>Lecanicillium lecanii</i>	WP	83.62de	3620d
<i>L. lecanii</i>	Oil	73.02e	3330d
<i>Bacillus thuringiensis</i>	WP	104.48cd	2115e
<i>B. thuringiensis</i>	Oil	110.86cd	1955e
SEm ±		8.60	101.24
CV %		12.03	3.93

### Half-life

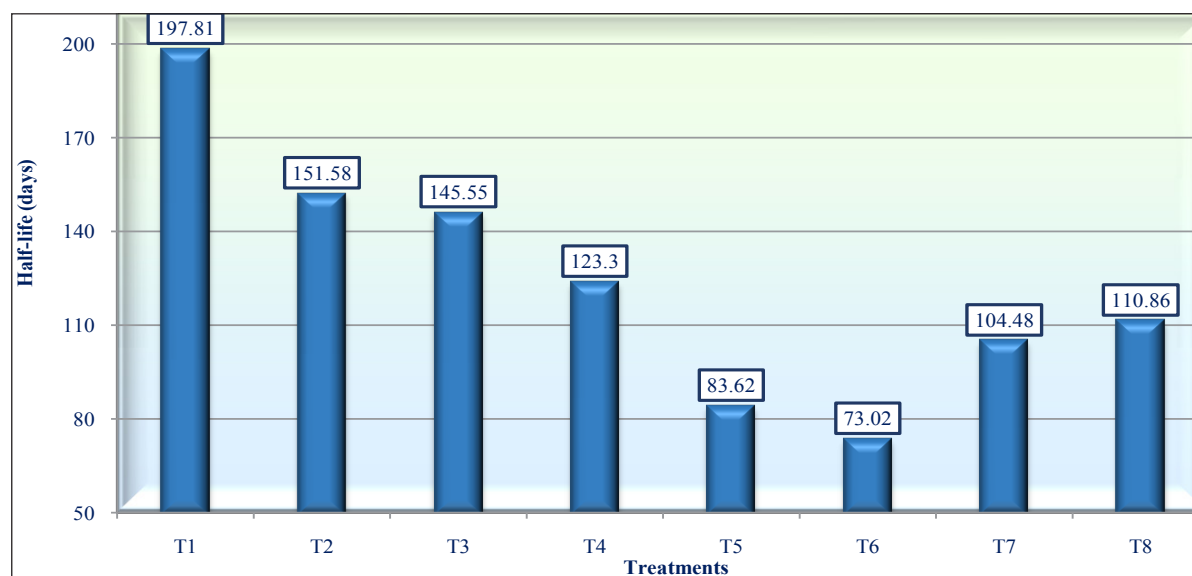
The time in terms of days required to reduce the mortality to half of its initial mortality is considered

as mortality half-life. Among the biopesticides evaluated, half-life ranged from 73.02 to 197.81 days among various evaluated biopesticides (Table 1).

*Beauveria bassiana* WP recorded half-life of 197.81 days which was highest and significantly different from other treatments. It depicts that the adult mortality reduced to 50 per cent after 197.81 days in case of *B. bassiana* WP. The treatments of *B. bassiana* oil, *Metarhizium anisopliae* WP and *M. anisopliae* oil with half-life of 151.58, 145.55 and 123.30 days, respectively were on par. There were no significant differences between the half-life values of *Bacillus thuringiensis* oil (110.86 days), *B. thuringiensis* WP (104.48 days) and *Lecanicillium lecanii* WP (83.62 days). Significantly lowest half-life was recorded in *L. lecanii* oil (73.02 days) and hence less effective.

### Gross Persistency

Based on gross persistency values presented in Table 1 and depicted in Fig. 2. *B. bassiana* WP was found significantly superior than rest of the treatments with gross persistency of 7115 followed by *B. bassiana* oil (6595). The gross persistency of *M. anisopliae* WP and *M. anisopliae* oil are found at par significantly with 5545 and 5380, respectively. *L. lecanii* WP (3620) and *L. lecanii* oil (3330) exhibited same gross persistency significantly. Among the eight treatments tested for gross persistency, significantly lowest gross persistency was recorded

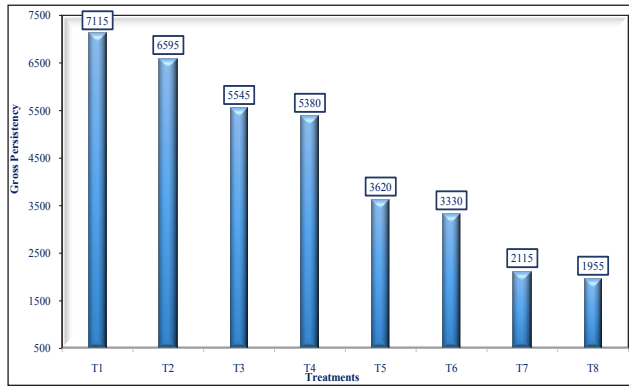


T1: *Beauveria bassiana* WP; T2: *Beauveria bassiana* oil; T3: *Metarhizium anisopliae* WP; T4: *Metarhizium anisopliae* oil; T5: *Lecanicillium lecanii* WP; T6: *Lecanicillium lecanii* oil; T7: *Bacillus thuringiensis* WP; T8: *Bacillus thuringiensis* oil.

**Fig. 1:** Half-life of biopesticides in treated chickpea seeds



by *B. thuringiensis* oil (1955) which was however at par with its WP formulation (2115).



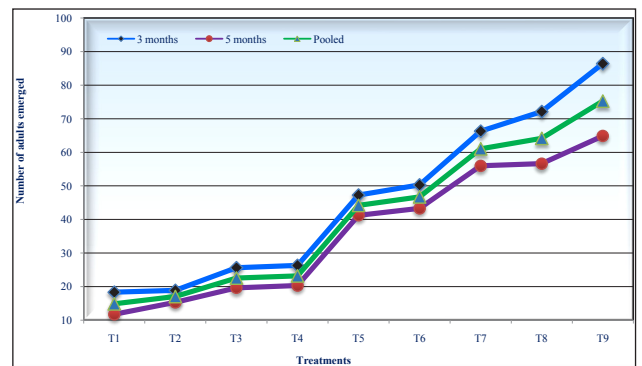
T1: *Beauveria bassiana* WP; T2: *Beauveria bassiana* oil; T3: *Metarhizium anisopliae* WP; T4: *Metarhizium anisopliae* oil; T5: *Lecanicillium lecanii* WP; T6: *Lecanicillium lecanii* oil; T7: *Bacillus thuringiensis* WP; T8: *Bacillus thuringiensis* oil.

Fig. 2: Gross persistency of biopesticides in treated chickpea seeds

### Evaluation Based on Population Growth

The data on number of adults developed after 3 and 5 months from the initial release of ten adults of *C. maculatus* for 5 days are presented in Table 2 and Fig. 3. The data developed after three months of storage revealed all biopesticidal treatments in WP and oil formulations were found significantly superior to control in reducing adult emergence of *C. maculatus*.

The data on number of adults emerged after 3 months of storage (Table 2 and Fig. 3) revealed that all treatments were found significantly superior over control in reducing population. Significantly least number of adults emerged out in the treatment of *B. bassiana* WP (18.35) after 3 months of storage in chickpea. 18.89 adults emerged in *B. bassiana* oil which was at par with *M. anisopliae* WP (25.66). However, *M. anisopliae* oil (26.32) was recorded at par with *M. anisopliae* WP. *L. lecanii* WP (47.27) stood next followed by its oil formulation (50.30). Significantly higher adult emergence was found in *B. thuringiensis* oil (72.14) at par with *B. thuringiensis* WP (66.33).



T1: *Beauveria bassiana* WP; T2: *Beauveria bassiana* oil; T3: *Metarhizium anisopliae* WP; T4: *Metarhizium anisopliae* oil; T5: *Lecanicillium lecanii* WP; T6: *Lecanicillium lecanii* oil; T7: *Bacillus thuringiensis* WP; T8: *Bacillus thuringiensis* oil.

Fig. 3: Population growth in biopesticides treated chickpea seeds after three and five months of storage

Table 2: Effect of biopesticides as grain protectant based on population growth during storage in chickpea

Treatments	Formulation	No. of adults emerged after storage		
		3 months	5 months	Pooled
<i>Beauveria bassiana</i>	WP	4.40a (18.35)	3.57a (11.77)	3.99a (14.89)
<i>B. bassiana</i>	Oil	4.46ab (18.89)	4.04ab (15.32)	4.25a (17.06)
<i>Metarhizium anisopliae</i>	WP	5.16bc (25.66)	4.55bc (19.66)	4.86b (22.56)
<i>M. anisopliae</i>	Oil	5.23c (26.32)	4.62c (20.33)	4.93b (23.23)
<i>Lecanicillium lecanii</i>	WP	6.99d (47.27)	6.50d (41.26)	6.72c (44.22)
<i>L. lecanii</i>	Oil	7.16d (50.30)	6.66d (43.32)	6.91c (46.75)
<i>Bacillus thuringiensis</i>	WP	8.21e (66.33)	7.55e (55.99)	7.88d (61.05)
<i>B. thuringiensis</i>	Oil	8.55e (72.14)	7.60ef (56.64)	8.07d (64.16)
Control	—	9.35f (86.44)	8.12f (64.90)	8.73e (75.30)
S. Em. ± Treatment (T)		0.23	0.16	0.01
Period (P)		—	—	0.07
T × P		—	—	0.20
C.V. %		6.07	4.79	5.55

Note: 1. Figures in parentheses are retransformed values, those outside are  $\sqrt{x+1}$  transformed values.  
 2. Treatment means with letter(s) in common are non-significant at 5 % level by DNMRT at 5% level.





The population growth after 5 months revealed lowest adult emergence in *B. bassiana* WP (11.77) which was at par with its oil formulation (15.32). Both WP (19.66) and oil (20.33) formulations of *M. anisopliae* gave significant efficacy towards population growth. Two interns gave equal adult emergence were *L. lecanii* WP (41.26) and *L. lecanii* oil (43.32). However, *M. anisopliae* WP was found at par with *B. bassiana* oil. Higher adult emergence was recorded in *B. thuringiensis* oil (56.64) at par with *B. thuringiensis* WP (55.99). Among all, population growth after 5 months of storage *B. thuringiensis* oil gave highest emergence proving it least effective.

Pooled data (Table 2 and Fig. 3) showed all treatments (14.89 to 64.16 adults) differed significantly from control (75.30 adults). Least adults were recorded in *B. bassiana* WP (14.89) which was at par with its oil formulation (17.06). Both WP (22.56) and oil (23.23) formulations of *M. anisopliae* gave significant efficacy towards population growth. Two interns *L. lecanii* WP (44.22) and *L. lecanii* oil (46.75) gave equal adult emergence. Significantly higher adult emergence was recorded in *B. thuringiensis* oil (64.16) at par with *B. thuringiensis* WP (61.05). Under present investigations on efficacy of biopesticides based on population growth *B. bassiana* WP was found superior over other treatments.

Different biopesticides under investigation recorded 73.02 to 197.81 days half-life. The half-life value was 197.81 days in *B. bassiana* WP and was found highly effective. The values ranged from 123.30 to 151.58 days in *M. anisopliae* oil, in *M. anisopliae* WP. Similarly, *B. bassiana* WP was found superiorly effective by recording gross persistency in the range of 7115. It was followed by *B. bassiana* oil and *M. anisopliae* WP which in turn was at par with *M. anisopliae* oil with gross persistency ranging between 6595 and 5380. Based on the population growth of *C. maculatus* after three and five months of storage, the least number of adults were recorded in *B. bassiana* WP (14.89) which was at par with *B. bassiana* oil formulation (17.06). It also depicted that WP formulations were comparatively effective than respective oil formulations for reducing the adult emergence effectively.

Somayeh *et al.* (2013) evaluated the virulence of ten fungal isolates of entomopathogenic fungi, *B. bassiana* and *M. anisopliae* against third instar larvae of Mediterranean flour moth, *E. kuehniella* Zeller

and reported that the mortality increased with the increase of exposure interval. Shaik (2015) reported that among different bio pesticide formulations evaluated for their efficacy on the longevity of pulse beetle, WP formulation of *M. anisopliae* and *B. thuringiensis* greatly affect the longevity of pulse beetle with average life span (1.67 days) followed by WP formulation of *V. lecanii* where the life span is 2.33 days. *B. bassiana* recorded the highest mortality among different biopesticides evaluated which is in accordance with the findings of Mannino *et al.* (2019) who reported that the potential advantage of *B. bassiana* over other EPFs due to the possibility to infect through both routes, oral and cuticular since it has a major number of shared genes with the other non-fungal pathogens that infect orally, such as *B. thuringiensis*. Better performance of *B. bassiana* over *M. anisopliae* was due to expression of proteins with a higher wide variability of molecular mass compared to latter EPF (Andre *et al.* 2007). The limited efficacy exhibited by *B. thuringiensis* is due to the limited toxicity showed by parasporal crystals of *Bt* (Lopez-Meza and Ibarra, 1996 and Vilas *et al.*, 1996). Biopesticides can be effectively used for the management of pulse beetles in storage and the present experiment provides future prospects for storage pest management studies.

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# Phenotypic Correlation Between Morphological and Yield Related Traits of Rice (*Oryza sativa* L.)

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## ABSTRACT

Rice is known as major staple food crop in India by providing food to more than 70 per cent population. An experiment was carried out in Alpha lattice design with two replications at ICAR-Indian Institute of Rice Research (ICAR-IIRR). An experiment was conducted during *kharif* season of year 2019 to study correlation and path coefficient analysis in 152 rice germplasm lines were collected. Analysis of variance showed significant differences among genotypes for all the 10 traits studied. Correlation studies revealed that grain yield per plant had positive and significant association plant height at both genotypic and phenotypic levels. Hence, these characters should be given due consideration while applying selection for increasing yield. Results of path analysis indicated that the maximum direct effects for plant height, tiller number per plant, no. of unfilled grains per panicle, panicle weight as well as appreciable indirect effects were exerted by days to 50% flowering, effective number of tillers, test weight, towards grain yield per plant. Therefore, selection pressure imposed on these traits would bring improvement in grain yield of rice.

## HIGHLIGHTS

- More number of tillers, high test weight leads to increases in yield.
- Yield related traits of rice.
- One fifty two genotypes for yield traits.

**Keywords:** Rice, Path Coefficient Analysis, Yield

Rice (*Oryza sativa* L.) is one of the important staple food crops with an annual production of 759.6 million tonnes (FAOSTAT, 2018). Rice feeds more than 70 percent population in India and contributes especially towards the global food security. However, rapidly increasing population has forced us to look for another quantum jump in rice production. Yield, being a complex trait, is composed of several components some of which affect the yield directly, while other affect indirectly. Hence, knowledge of association between Morphological, yield and its

components is necessary. Selection directly based on the performance of seed yield, may not be very effective but selection based on its component characters would prove more effective as reported in other plants (Fisher 1918). Correlation studies would provide estimates of degree of association between

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seed yield, its various components and also among the components. Although studies on correlation are helpful in determining the components of complex characters like seed yield, these estimates do not provide an exact information about the relative magnitude of direct and indirect influences of each of the component character on seed yield. In this context Wright (1921) proposed estimation of path coefficient analysis as an important tool in partitioning the correlation coefficient into two parts, direct and indirect effects which will be useful in identifying biometrical characters to achieve desirable goal. Therefore, path-coefficient analysis is important to elucidate the intrinsic nature of association of component traits by determining the direct or indirect contribution of these traits to yield.

## MATERIALS AND METHODS

One hundred and fifty two (152) rice germplasm lines collected from all over India and Philippines were evaluated for yield and component traits during *Kharif* 2019 in Alpha lattice design with two replications at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad.

Thirty days old seedlings were transplanted by adopting a spacing of 15 cm between plants and 20 cm between rows. Recommended agronomic and plant protection measures for raising a healthy nursery and main crop were taken up during the experiment.

Replication-wise data on the basis of five randomly taken competitive plants were recorded on following traits: viz., days to fifty per cent flowering (DFF), Total number of tillers per plant (TNT), effective number of tillers (ENT), plant height (PH) (cm), number of filled grains (NFG), number of un filled grains (NUFG), panicle weight (PW) (g), panicle weight (PL) (cm), test weight (TW) (g), and yield per plant (YPP) (g). The mean of five plants for each metric trait was considered for statistical analysis using SAS software. The analysis of variance (ANOVA) was done on the basis of model described by Cochran and Cox (1950) for Alpha lattice design. The genotypic and phenotype variances were calculated as per the formulae proposed by Burton and Devane (1953). The replicated data were subjected to statistical analysis. The estimates of covariances were worked out as per Singh and

Choudhary (1985). The estimates of covariances and variances were utilized in computing genotypic and phenotypic path coefficient was worked out as suggested by Wright (1921).

## Path coefficient analysis

The genetic architecture of grain yield is based on the overall net effect produced by various yield components interacting with one another. The association of different component characters among themselves and with yield is quite important for devising an efficient selection criterion for yield. Correlation gives only the relation between two variables, whereas path coefficient analysis allows separation of the direct effect and their indirect effects through other attributes by partitioning the correlations (Wright 1921). Based on the data recorded on the genotypes in the present investigation, the phenotypic correlations coefficients were estimated to determine direct and indirect effects of yield and contributing characters. The results for phenotypic path coefficient analysis for yield and its component traits are presented in table 1.

For the improvement of crop for higher yield prior to any breeding program, it is commandment to obtain information regarding the inter-relationship of different characters with yield and among themselves, since it facilitates the quicker assessment of high yielding and better performing genotypes in selection programmed. The phenotypic correlation coefficients were estimated among 10 traits of 152 rice germplasm, to find out the association of grain yield and other yield contributing characters (Table 1).

## RESULTS AND DISCUSSION

### Days to 50% flowering

Days to 50% flowering had negative and direct effect (-0.067) on single plant yield at phenotypic level in 2019. It had indirect negative effects on single plant yield through total no. of tillers (-0.005), productive tiller number (-0.006), panicle length (-0.004), panicle weight (-0.014), no. of un filled grains (-0.005), yield per plant (-0.004). It had indirect positive effects on single plant yield through, plant height (0.003) and test weight (0.004) at phenotypic level. Yadav *et al.* (2011) reported Days to 50% flowering exhibited

**Table 1:** Direct Indirect Effect Phenotypically between Morphological and yield traits in 2019

	<i>DFE</i>	<i>PH</i>	<i>TNT</i>	<i>ENT</i>	<i>NFG</i>	<i>NUFG</i>	<i>PW</i>	<i>PL</i>	<i>TGW</i>	<i>YPP</i>
<i>DFE</i>	<b>-0.067</b>	0.003	-0.005	-0.006	0	-0.00542	-0.01	-0.004	0.004	-0.004
<i>PH</i>	-0.001	<b>0.022</b>	-0.000	-0.001	0.0047	0.001694	0.00	0.0029	0.008	-0.008
<i>TNT</i>	0.0039	-0.00	<b>0.045</b>	0.0415	0.0034	0.00252	-0.00	-0.008	-0.005	0.004
<i>ENT</i>	-0.009	0.005	-0.098	<b>-0.107</b>	-0.011	-0.00556	0.00	0.0163	0.012	-0.013
<i>NFG</i>	0	-0.03	-0.012	-0.017	<b>-0.162</b>	-0.05459	-0.07	-0.012	0.020	-0.015
<i>NUFG</i>	0.0118	0.011	0.0081	0.0075	0.0492	<b>0.146</b>	0.02	0.0124	-0.015	0.013
<i>PW</i>	0.0345	0.033	-0.016	-0.012	0.0736	0.03007	<b>0.15</b>	0.0252	0.006	0.013
<i>PL</i>	0	0	0	0	0	0	0	0	0	0
<i>TGW</i>	0.0098	-0.00	0.0171	0.0174	0.0187	0.01537	-0.00	-0.002	<b>-0.145</b>	-0.000
<i>YPP</i>	-0.003	0.001	-0.005	-0.006	-0.004	-0.0046	-0.00	-0.004	-0.000	-0.05

\*Significance at 5% level, \*\*Significance at 1% level.

*DFE*- Days to 50% flowering, *TNT*- Tillers number per plant, *ENT*- Effective number of tillers, *PH*- Plant height, *NFG*- No. of filled grains, *NUFG*- No. of unfilled grains, *PW*- Panicle weight, *PL*- Panicle length, *TGW*- Test weight, *SPY*- Single plant yield.

direct negative effect. Gour *et al.* (2017) reported days to 50% flowering showed direct negative effect.

### Plant height

Plant height had positive and direct effect (0.022) on single plant yield at phenotypic levels in 2019. It had indirect negative effects on single plant yield through Days to 50% flowering (-0.001122), total no. of tillers (-0.000836), productive tiller number (-0.001166), yield per plant (-0.000836). It had indirect positive effects on single plant yield through, panicle length (0.00297), panicle weight (0.004774), no. of un filled grains (0.001694), no. of filled grains (0.00473) and test weight (0.0008) at phenotypic level.

Chakraborty *et al.* (2010) revealed the positive direct effect of plant height, on grain yield. Pankaj *et al.* (2010) revealed the direct positive effect of plant height at maturity on yield per plant at genotypic level. Ambili and Radhakrishnan (2011) reported high positive direct effect of plant height on grain yield, followed by total no. of tillers, number of productive tillers per plant, Panicle length, panicle weight, no. of filled grains, test weight. The high negative direct effect on yield was recorded for days to flowering. They concluded that yield of rice can be improved by selecting medium tall genotypes having more number of productive tillers per plant, higher straw yield and an optimum duration.

### Total no. of Tillers

Total no. of Tillers had positive and direct effect

(0.045) on single plant yield at phenotypic levels in 2019. It had indirect negative effects on single plant yield through plant height (-0.00171) panicle weight (-0.004815), panicle length (-0.00882), test weight (-0.00531). It had indirect positive effects on single plant yield through, Days to 50% flowering (0.003915), productive tiller number (0.04158), total no. of filled grains (0.00342), no. of un filled grains (0.00252), yield per plant (0.004995).

Saravanan and Sabesan (2009) revealed the estimates of direct effects for the number of tillers per plant with high direct effect on grain yield per plant, suggesting that the improvement in grain yield will be efficient if the selection is based on these component traits. Pankaj *et al.* (2010) revealed the direct positive effect of tillers per plant on yield per plant. Ravindra Babu *et al.* (2012) reported that number of tillers per hill exhibited maximum direct effect on grain yield per hill at phenotypic levels. Aditya and Anuradha (2013) revealed that tillers per plant exhibited positive direct effect on grain yield.

### Effective no. of tillers

Productive no. of tillers had negative and direct effect (-0.107) on single plant yield at phenotypic levels in 2019. It had indirect negative effects on single plant yield through Days to 50% flowering, (-0.009844), productive tiller number (-0.098868), no. of un filled grains (-0.01177), yield per plant (-0.013161). It had indirect positive effects on single plant yield through, plant height (0.005671), panicle weight (0.008667), panicle length (0.016371), test

weight (0.01284). Khan *et al.* (2009) reported negative direct effect of number of productive tillers per plant on grain yield. Sarawagi *et al.* (2016) reported that effective tillers per plant showed positive direct effect on grain yield per plant.

### **Number of filled grains**

Number of filled grains had negative and direct effect (-0.162) on single plant yield at phenotypic levels in 2019. It had indirect negative effects on single plant yield through plant height (-0.03483) panicle weight (-0.07695), panicle length (-0.012636), no. of un filled grains (-0.054594), yield per plant (-0.015714). no. of tillers (-0.012312) productive tiller number (-0.01782). It had indirect positive effects on single plant yield through, test weight (0.020898). Similar results were reported by Ashok *et al.* (2016), and Nithya *et al.* (2020)

### **Number of unfilled grains**

Number of unfilled grains had positive and direct effect (0.146) on single plant yield at phenotypic levels in 2019. It had indirect negative effects on single plant yield through test weight (-0.015476). It had indirect positive effects on single plant yield through, Days to 50% flowering, (0.011826) plant height (0.011242), no. of tillers (0.008176) productive tiller number (0.007592), total no. of filled grains (0.049202), panicle weight (0.028324), panicle length (0.01241), yield per plant (0.013432). Similar results found by Parimala *et al.* (2020).

### **Panicle weight**

Panicle weight had positive and direct effect (0.155) on single plant yield at phenotypic levels in 2019. It had indirect negative effects on single plant yield through, total no. of tillers (-0.016585) productive tiller number (-0.012555). It had indirect positive effects on single plant yield through, Days to 50% flowering, (0.034565) plant height (0.033635), total no. of filled grains (0.073625), no. of un filled grains (0.03007), panicle length (0.025265), test weight (0.0062), yield per plant (0.01395).

### **Panicle length**

Panicle length had positive and direct effect (0.046) on single plant yield at phenotypic levels in 2020. It had indirect negative effects on single plant yield

through, total no. of tillers (-0.005382) productive tiller number (-0.00736), test weight (-0.000046). It had indirect positive effects on single plant yield through, Days to 50% flowering (0.00506), plant height (0.002576), total no. of filled grains (0.006486), no. of un filled grains (0.005152), yield per plant (0.002208), panicle weight (0.007498),

Awaneet and Senapati (2013) revealed that panicle length exhibited positive direct effect on grain yield, while 1000 grain weight exhibited negative direct effect on grain yield. Chouhan *et al.* (2014) evaluated thirty five rice accessions to assess their character association and path analysis for grain yield and yield attributing traits. Path analysis revealed positive direct effects of traits panicle length on yield per plant. Dhurai *et al.* (2016) revealed that panicle length exhibited high positive direct effects on grain yield. Harsha *et al.* (2017) revealed that at phenotypic level panicle length direct positive effect

### **Thousand test weight**

Thousand test weight had negative and direct effect (-0.145) on single plant yield at phenotypic levels in 2019. It had indirect negative effects on single plant yield through plant height (-0.005655) panicle weight (-0.0058), panicle length (-0.00232), test weight (-0.00087), yield per plant (-0.00087). It had indirect positive effects on single plant yield through, total no. of tillers (0.01711), productive tiller number (0.0174), total no. of filled grains (0.018705), no. of un filled grains (0.01537). Chakraborty *et al.* (2010) revealed the positive direct effect of 1000-grain weight on grain yield. Harsha *et al.* (2017) revealed that at phenotypic level, test weight showed direct positive effect.

### **Yield per plant**

Yield per plant had negative and direct effect (-0.05) on single plant yield at phenotypic levels in 2019. It had indirect negative effects on single plant yield through, Days to 50% flowering, (-0.0033) panicle weight (-0.0045), panicle length (-0.00425), test weight (-0.0003) total no. of tillers (0.00555), productive tiller number (-0.00615), total no. of filled grains (-0.00485), no. of un filled grains (-0.0046), yield per plant (-0.05). It had indirect positive effects on single plant yield through, plant height (0.0019).



The data showed that correlation at genotypic and phenotypic levels had the same trend. The values of genotypic correlation coefficients were higher than those of their respective phenotypic correlation coefficients in most of the cases, suggesting that there was a strong and inherent association between two characters. In some cases, however, the phenotypic correlation was slightly higher than their genotypic counterpart, which implied that the non-genetic causes inflated the value of genotypic correlation because of the influence of environmental factors.

## CONCLUSION

Thus, it concludes that path analysis based on phenotypic correlation showed high direct effect of plant height, tiller number per plant, no. of unfilled grains per panicle, panicle weight as well as appreciable indirect effects were exerted by days to 50% flowering, effective number of tillers, test weight, towards grain yield per plant. Revealing scope for considering these characters for imposing selection pressure for bringing out an improvement in rice yield. On the basis of all the above findings, it can be concluded that, while imposing selection for genetic improvement of grain yield in rice.

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# Utilization of Bamboo and Wooden Resources for Development of Contemporary Products: An Approach for Climate Change Mitigation and Revenue Generation

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## ABSTRACT

Globally, there is a rising demand for plant based contemporary products in urban & peri-urban areas. Additionally, the Government of India (GoI) & Indian Council of Agricultural Research, New Delhi has also emphasized skill development among students and farmers through Hands-on-Trainings (HoTs) for growth of bamboo & wooden entrepreneurship in the country. The Navsari Agricultural University, Navsari, Gujarat was awarded with an innovative project on Secondary Agriculture through NAHEP - CAAST (Center for Advanced Agricultural Science & Technology). Utilization & value addition of plant resources (*viz.*, waste branches, roots, stumps, bamboo, seeds, cones, inflorescences) sustainably is one of the unique concepts of this project. This activity also finds place in the Vocal for Local concept of GoI, wherein local products are promoted at the global level through various government interventions. Preparation of contemporary products by wood & bamboo resources is one such activity of the project which has yielded excellent outcomes through design development & providing HoTs to farmers & students. There is ample scope for entrepreneurship in the field of contemporary products development using bamboo & wooden raw materials including their waste. This entrepreneurship is characterized by novelty & to flourish in a demand based environment to produce novel products. Over and above 60 innovative as well as contemporary bamboo & wooden products have been prepared, six pamphlets of success stories as well as one training manual on wooden decorates were published at NAU, Navsari under the aegis of NAHEP-CAAST sub-project. Therefore, rural farming communities & youngsters can involve themselves in preparing contemporary wood & bamboo products which may fetch premium rates in the market. Preparation of contemporary products using wood and bamboo can provide not only meaningful revenue generation & employment opportunities to the needy by making best use of locally available plant resources but also locks carbon for a long time hence development of contemporary products using bamboo & wooden resources an approach for climate change mitigation and revenue generation.

## HIGHLIGHTS

- ① Utilization and value addition of plant resources (*viz.*, waste branches, roots, stumps, bamboo, seeds, cones, inflorescences) sustainably is one of the unique concepts of this project.
- ② Six pamphlets of success stories as well as one training manual on wooden decorates were published at NAU, Navsari.

**Keywords:** Contemporary products, climate change, wealth from waste, carbon sink

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Secondary Agriculture is regarded as the sunrise sector of the Indian economy owing to its immense untapped potential. The Secondary Agriculture sector has a major role to play in employment generation, poverty alleviation and product diversification including utilization of woody/bamboo biomass. Among secondary agricultural products, Non-timber forest resources and woody/bamboo biomass also play a vital role in the forestry sector (Anonymous 1972). There is a growing demand for contemporary products development in urban & peri-urban areas (Sankanur *et al.* 2022a). Additionally, the Government of India (GoI) & Indian Council of Agricultural Research (ICAR), New Delhi has also accentuated skill development among students and farmers through Hands-on Trainings (HoTs) for growth of bamboo & wooden entrepreneurship through craftsmanship in the country. ICAR-NAHEP, New Delhi has granted a Center for Advanced Agricultural Science & Technology (CAAST) on Secondary Agriculture to the Navsari Agricultural

University (NAU), Navsari, Gujarat. Utilization & value addition of plant resources sustainably is one of the unique concepts of this project (Sankanur *et al.* 2020). This activity also finds place in the Vocal for Local concept of GoI, wherein local products are promoted at the global level through various government interventions. Preparation of contemporary products is solitary of the ways to get better income and uplift living standards (Sankanur *et al.* 2022b). The forest fringe villagers/ farmers who adopt agroforestry systems may take this benefit, especially women farmers or house wives for preparation of contemporary products from wood/bamboo and other resources by single or SHGs (Self-Help-Groups). This particular manuscript provides a platform to address the utilization and value addition from both bamboo and wooden resources utilizing tools, equipment & machineries.

By looking into these production potentials of contemporary products from bamboo & wooden resources use of each of these tools, equipment & machineries are elaborated as given in Table 1.

**Table 1:** List of tools and machineries used for production of sculptures from wood/bamboo

Sl. No.	Name of the equipments	Uses of equipments
1	Seasoning cum preservation vacuum pressure equipment	For preservation treatment and seasoning of wood / wooden log / bamboo / canes
2	Wood chipper	Machine converts larger samples of wood and wood products into smaller wood chips
3	Wood grinder (Fine grinding machine/ Hammer Mill)	Machine converts wood chips into fine powder for particle board or fibre board making
4	Circular saw (different size)	Demonstration of cross cutting of wood/stem
5	Power chain saw (different size)	Demonstration of cross-cutting of wood/stem
6	Agarbatti Making Machine	Preparation of Agarbatti of 80-90 kilo grams weight production per 8 Hour (depends on feeder).
7	Charcoal making machine	To convert biomass (agriculture wastes or forestry wastes that are rich in lignin, cellulose, hemicellulose, such as straw, sawdust, rice husk, fruit hush, coconut shell, palm shell, tree bark, small branches, logs, <i>etc</i> ) into charcoal.
8	Briquette making machine	To convert forest waste (Saw dust, sander dust, wood chips & shavings, tree bark & twigs, pine needles, wild grass, shrubs, bamboo leaves, veneer waste, wood peeling waste) into biomass briquette without any need of binder or adhesive.
9	Rotary sander	Wood polishing or removing rust. The rotating pad enables to quickly prepare large surface for more advanced prepping.
10	Hot press	Mainly used to fabricate hard boards.
11	Turning lathe machine	To rotate a work/wood piece about an axis of rotation to perform various operations such as cutting, sanding, knurling, drilling, deformation, facing and turning, with help of tools that are applied to the workpiece to create an object with symmetry about that axis.
12	Boring machine	Used for producing smooth and accurate holes in a workpiece by enlarging existing holes with a bore, which may bear a single cutting tip of steel, cemented carbide or diamond or maybe a small grinding wheel

13	Tenoning machine	Used for making tenon for furniture, wood door, easy operation
14	Surface planer cum Thickness planer (Combi-planner)	This machine used for an active role in applications like used for grooving, sawing, planning and designing to prepare contemporary products from Bamboo and Wood
15	Band Saw	Used principally in woodworking, metalworking, and lumbering, but may cut a variety of materials for R & D to prepare novel products from Bamboo and Wood
16	Bamboo toothpick machines	Bamboo Toothpick Processing Machine is mainly used to process bamboo into small toothpicks. Final product with diameter 2.2 mm and the length 65mm with two sharpened ends. The capacity is 600000 pcs/8 hours. Used for R & D on bamboo-based products preparation.
17	Bamboo bending machine	Useful for <i>bamboo</i> furniture, construction, fencing, artisan works, Used for Research & Development to bamboo based modern products preparation
18	Kulfi stick making machine	Used for is mainly used to process bamboo into small Kulfi sticks and R & D for preparation of innovative products

**Table 2:** List of tools/ equipment's/materials required for preparation of contemporary products

Hammers	Gas torch burners
Pliers	Wood Carving tools
Drills	Acrylic paint for wood
Sand papers (60-180 grit)	Thin metal plates
Nails	Eye wears
Adhesive	Small Hand saws
Rotary sanders	Varnishes
Smoother	Wooden glues
Paint brushes	Clay Powder
J type hooks	

All these tools / materials are having its potential scope for production contemporary products from wood/bamboo raw material including their wastes and other resources like branches, harvested tree roots, *etc.*

## METHODOLOGY

These contemporary products are prepared by hand and/ or machine using wood/bamboo as well as wastes from wooden samples/blocks, inflorescences, branches, flowers, twisted stems, roots and it requires an art and skill for preparation of attractive/fancy contemporary products. In the rural areas, contemporary products and other household utensils may be carved out of wood in different shapes and styles.

## RESULTS

**Outcome, output & impact of activity:** Earlier tribal artisans and local farmers of Ambapada village used to prepare only limited range of traditional products and now after two hands on trainings

in the project, they are able to prepare new range of contemporary products on their own *viz.*, table lamp, bamboo candle, bamboo lamp, climber sculpture, tree sculpture, garden tree sculpture, hanging tree sculpture, rakhi's, bamboo candle stands, contemporary wall clocks, contemporary chairs, contemporary garden chairs, *etc.* (Sankanur *et al.* 2022c; Sankanur *et al.* 2022d; Sankanur *et al.* 2022e; Sankanur *et al.* 2022f; Sankanur *et al.* 2022g). It is only due to interventions of the NAHEP-CAAST sub-project. Moreover, about 73 *Kotawali* tribal artisans of Waghai, Dangs, Gujarat were also trained which yielded change in their livelihood by earning 20 to 40K additional income per annum. In our unit, four local peoples were trained by providing skills for preparation of wooden based articles and now they are acting as master trainers for trainees. They have already prepared more than 60 bamboo & wooden contemporary products with the help of forestry faculty. We also prepared six pamphlets of success stories and published one training manual on wooden contemporary products at NAU, Navsari. In addition to this, contemporary products developed under NAHEP-CAAST sub-project also includes various designs of climber flowering plants, key chain hangers, stump contemporary products, corner contemporary products, table contemporary products, room lamps, table lamps, tea coasters, bamboo waterfall, tree machanas, farm houses, multi-stage flowering pot plants, peacocks, ducks, wall hangings, wooden desk organizers, wide range of pen stands, tea trays, boats, carts, aircrafts, pot stands, eco-friendly rakhi's, bamboo candle stands, Bamboo acoustic mobile speaker, Small bird play gyms, contemporary wall clocks, contemporary wall



racks, contemporary chairs, contemporary garden chairs *etc.* (Sankanur *et al.* 2019a; Sankanur *et al.* 2019b & Sankanur *et al.* 2022h).

## CONCLUSION

It is found that wood/bamboo resources such as branches, roots & stumps left out from the forests, plantations, agroforestry landuse systems &/or saw mills are considered as waste materials & these may be utilized for development of value-added attractive products *i.e.* wealth from waste. India has the second largest in bamboo resources in the world and many peoples are dependent on them for their livelihood. Now there is a need to promote the utilization of bamboo & wooden waste to the best possible extent. The potential of bamboo and wood resources is very high but it remains largely unrealized in India. By making different contemporary products and its marketing forest/plantation/agroforestry waste can be better utilized. A large number of employment opportunities can be tapped by preparation & marketing of contemporary products. Preparation of contemporary products is solitary of the ways to get better the income and uplift living standards of people involved in it. The tribes / artisans /forest fringe villagers/ farmers adopt agroforestry systems may take this benefit, especially women farmers or house wives/youth for preparation of contemporary products from wood/bamboo and other resources by single or SHGs (Self-Help-Groups).

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# Estimation of Genetic Parameters of Willow (*Salix* spp.) Clones for Leaf Traits

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## ABSTRACT

The present investigation was carried out to analyze the leaf traits viz., total leaf length (cm), midrib length (cm), petiole length (cm), petiole appendages, maximum leaf width (cm) and number of teeth per cm on leaf margins for the relative performance of 15 clones of willow (*Salix* spp.). All the studied leaf parameters showed significant variations among the clones. Based on the mean values of the studied leaf traits five clones namely UHFS06, UHFS008, UHFS013, UHFSP1 and UHFSP4 performed better than other clones. Among the traits, maximum leaf width showed maximum value of genotypic (18.91%) and phenotypic (24.78%) coefficient of variation. The magnitude of PCV was found to be slightly higher than GCV. Maximum heritability was recorded for number of teeth per cm on leaf margins *i.e.*, 64.75 percent whereas maximum genetic gain (29.72%) was recorded for maximum leaf width. The results reveal that the clones selected based on these traits can be further utilized in tree improvement and breeding programs.

## HIGHLIGHTS

- ① Study was carried out in nursery conditions where clones were planted in Randomized Block Design with the spacing of 30 cm × 40 cm using 3 replications.
- ① Significant differences was observed for all the studied leaf traits.
- ① Mean values, genotypic coefficient of variability, phenotypic coefficient of variability, heritability, genetic advance and genetic gain was calculated.
- ① Clones with better performance will be further utilized for tree improvement and breeding programs.

**Keywords:** Leaf traits, *Salix* spp., GCV, PCV, Heritability, Genetic gain

Willows, or the genus "*Salix*", members of the family Salicaceae, a group of flowering plants that diversify at genetic, species and ecological level with 450-520 species worldwide (Wu *et al.* 2015). This genus is said to have originated in East Asia between 20° and 40°N; it mostly inhabits the Southern, Southwestern China and Northern Indo-China Peninsula regions of the continent and constitutes over 375 species, or about 71.29 percent (approx.) of all *Salix* species worldwide (Fang 1987). Willows are fast-growing, short rotation trees with young stems that are both flexible and strong. Willow leaves are simple, typically long, thin and oblong or lanceolate with smooth or serrated edges. They are rarely opposite and are placed alternatively on the branches.

Concerned species is a multipurpose species that produces fodder, fuelwood and small timber. The primary sources of fodder for cattle, sheep and goats are its leaves and bark (Rawat *et al.* 2006). Willows are highly productive, frequently producing 20 or 25 shoots from a single coppice stool (Sennerby-Forsse *et al.* 1994). They can grow up to 4 m tall in the first three years of coppicing and can produce up to 20 ODT ha<sup>-1</sup> yr<sup>-1</sup> (Bassam 1998). Sports item *i.e.* cricket bats etc. are ideally made of willow wood

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(Bhat 2004). Natural hybridization has produced a variety of distinct forms for *Salix* in the Himalayan region, each with its own habits, characteristics for use, and added value (Biswas and Hussain 2008). Willow improvement works initiated with the introduction of willow clones and their screening in the nursery (Singh *et al.* 2012c; Sharma *et al.* 2014), field testing of better clones raised in the university (Sharma *et al.* 2011) and farmer’s field (Sharma *et al.* 2015b). From the clones of willow, hybrids were produced (Choudhary *et al.* 2013). The evaluation and selection of willow hybrids (Sharma *et al.* 2018) have been completed. This study seeks to analyse the relative performance of willow clones on the basis of leaf traits.

## MATERIALS AND METHODS

The experiment was carried out at Naganji nursery, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (HP) in the year of 2021. The nursery site is situated between 30°51’N latitude and 76°11’E longitude in the North-West of Himalaya at an elevation of 1200 m above mean sea level. The experimental area has mild slopes towards the South-Eastern aspect and is hilly with elevations and depressions. The selected clones were introduced from the local germplasm. However, due to large number of clones and limited availability of uniform land in nursery, 15 ramets of each clone were randomly planted in single replication with

the spacing of 30 cm × 40 cm using three replications in Randomized Block Design (RBD) under the nursery conditions.

The observations were made on fully expanded matured leaves in the middle of the main shoot in the middle of the growing season, from mid-July to mid-August. With the help of a measuring scale, the total leaf length, midrib length, petiole length, and maximum leaf width were all measured and expressed in centimeters. The number of leaf teeth was counted from the leaf margin at the centre of the leaf blade within one centimeter, whereas the number of petiole appendages was counted from the junction of the emergence of the leaf blade and petiole. For measurements of the total leaf length, midrib length, petiole length, petiole appendages, maximum leaf width, and number of leaf teeth per cm on the leaf margins, fifteen randomly selected leaves per clone have been used.

The analysis of variance (ANOVA) table was set up as follows:

$$Y_{ijk} = \mu + c_i + r_j + e_{ij}$$

$$i = 1, 2, \dots, c$$

$$j = 1, 2, \dots, r$$

Where,

$Y_{ijk}$  = phenotypic observation of  $i^{\text{th}}$  entry and  $j^{\text{th}}$  replication.

**Table 1:** Detail of willow clones used for the study

Sl. No.	Clone	Species	Source country/ originally developed
1	J799	<i>S. matsudana</i> × <i>S. alba</i>	UK/China
2	NZ1179	<i>S. matsudana</i> × <i>S. alba</i>	UK/Newsland
3	J194	<i>S. matsudana</i> × <i>S. arbutifolia</i> × <i>S. matsudana</i>	UK/China
4	Kashmiri	<i>S. alba</i> cv. <i>coerulea</i>	UK
5	SI-63-007	<i>S. alba</i>	Italy
6	NZ1140	<i>S. matsudana</i> × <i>S. alba</i>	UK
7	131/25	<i>S. babylonica</i> × <i>S. alba</i>	UK/Aregentina
8	AUSTREE	<i>S. alba</i> × <i>S. matsudana</i>	UK/Newsland
9	J172	<i>S. babylonica</i> × <i>S. alba</i> × <i>S. matsudana</i>	UK/China
10	<i>S. tetrasperma</i>	<i>S. tetrasperma</i> (♀)	Local selection
11	PN227	<i>S. matsudana</i>	Newsland
12	SE-69-002	<i>S. matsudana</i>	Italy
13	FLS	<i>S. tetrasperma</i> (♂)	Local selection
14	Majnu	<i>S. alba</i>	Local selection
15	J795	<i>S. matsudana</i> × <i>S. alba</i>	UK/China



$M$  = general mean of population

$c_i$  = effect of  $i^{\text{th}}$  clone

$r_j$  = effect of  $j^{\text{th}}$  replication, and

$e_{ij}$  = error component

Coefficient of variability (CV%) was calculated by the formula given by Burton and De Vane, (1953).

$$\text{PCV (\%)} = \frac{\sqrt{V_p}}{X} \times 100;$$

$$\text{GCV (\%)} = \frac{\sqrt{V_g}}{X} \times 100;$$

$$\text{ECV (\%)} = \frac{\sqrt{V_e}}{X} \times 100;$$

$$\text{CV (\%)} = \left( \frac{SD}{X} \right) \times 100$$

Where,

PCV = Phenotypic Coefficient of Variability

GCV = Genotypic Coefficient of Variability

ECV = Environment Coefficient of Variability

$\bar{X}$  = Population mean of character

$V_p$  = Phenotypic variance

$V_g$  = Genotypic variance

$V_e$  = Environmental variance

SD = Standard deviation

$\bar{X}$  = Population mean

Heritability ( $h^2$ ) in broad sense was calculated as suggested by Burton and De Vane (1953) and Johnson *et al.* (1955).

$$h^2 = \frac{V_g}{V_p} \times 100$$

Where,

$h^2$  = Broad Sense Heritability

$V_g$  = Genotypic variance

$V_p$  = Phenotypic variance

The expected genetic advance at 5 per cent selection intensity was calculated by the formula suggested by Lush (1940) and further used by Burton and De Vane (1953) and Johnson *et al.* (1955).

$$\text{Genetic advance (GA)} = \left[ \frac{V_g}{V_p} \right] \times (\sqrt{V_p}) \times K$$

Where,

$K = 2.06$  (Selection differential at 5 % selection intensity) (Allard 1960).

Genetic gain was calculated as suggested by Johnson *et al.* (1955).

$$\text{Genetic gain (\%)} = \frac{\text{Genetic advance}}{\bar{X}} \times 100$$

## RESULTS AND DISCUSSION

The data in the Table 2 depicted that there was significant variations for all the examined traits. Kashmiri clone recorded with maximum total leaf length (17.16 cm) and midrib length (15.96 cm) which was statistically at par with *S. tetrasperma* (16.51 cm and 15.02 cm respectively) and NZ1140 (14.73 cm and 13.75 cm respectively). However, minimum value was recorded for J172 (11.51 cm and 10.48 cm respectively). Maximum value for petiole length was recorded for *S. tetrasperma* (1.52 cm) and minimum value was recorded for SE-69-002 (0.83 cm). Maximum value for petiole appendages was recorded for SI-63-007 clone (2.53) and for maximum leaf width it was for clone 131/25 (2.72 cm). Whereas, petiole appendages and maximum leaf width recorded minimum value for Majnu clone (1.29 and 1.26 cm respectively). FLS clone was recorded maximum value for number of leaf teeth (5.82) and AUSTREE was recorded with minimum value (3.98). In contrary to our study, Singh *et al.* 2012 obtained significant differences among the leaf traits in nursery growth of willow.

Table 3 showed the values of genetic parameters for studied leaf traits of willow clones. The maximum coefficient of variability values i.e. GCV and PCV was recorded for maximum leaf width i.e. 18.91 % and 24.78 % respectively.

The magnitude of PCV values was slightly higher than the GCV values which suggested that the traits were highly influenced by the environment. Thakur *et al.* (2020) found similar results among the inter-specific progenies of *Bauhinia variegata*. The high heritability value was recorded for number of teeth per cm on leaf margins (64.75 %) which indicated that the traits is under the strong genetic control and the predominance of additive gene action for governing of traits among the clones whereas, low value was found for midrib length (42.32 %).

**Table 2:** Total leaf length, midrib length, petiole length, petiole appendages, maximum leaf width and number of teeth per cm on leaf margins of willow clones

Clones name	Total leaf length	Mid rib length	Petiole length	Petiole appendages	Maximum leaf width	No. of teeth per cm on leaf margins
J799	14.10	13.10	1.00	2.44	1.65	4.36
NZ1179	14.30	13.17	1.13	2.11	1.74	4.33
J194	13.57	12.34	1.23	2.16	1.73	4.71
Kashmiri	17.16	15.96	1.20	2.42	2.06	4.53
SI-63-007	12.04	11.01	1.02	2.53	2.50	4.22
NZ1140	14.73	13.75	0.98	2.04	1.86	4.51
131/25	13.71	12.59	1.12	2.07	2.72	4.47
AUSTREE	13.20	12.25	0.95	2.04	1.56	3.98
J172	11.51	10.48	1.03	1.93	1.72	4.24
S. tetrasperma	16.51	15.02	1.52	2.13	2.55	5.56
PN227	12.57	11.64	0.92	1.82	1.79	4.16
SE-69-002	13.19	12.36	0.83	1.73	2.07	4.47
FLS	12.53	11.65	0.87	2.07	1.81	5.82
Majnu	12.28	11.43	0.84	1.29	1.26	4.78
J795	13.27	12.28	0.99	2.20	1.66	4.80
Mean	13.64	12.60	1.04	2.07	1.91	4.60
SE	1.22	1.15	0.13	0.21	0.25	0.28
CD <sub>(0.05)</sub>	2.50	2.35	0.28	0.43	0.51	0.57
CV (%)	10.97	11.17	15.78	12.45	16.01	7.41

**Table 3:** Estimation of genetic parameters for leaf traits of willow clones

Leaf traits	Mean	Range	Coefficient of variation (%)		Heritability (%)	GA	GG (%)
			Genotypic	Phenotypic			
Total leaf length (cm)	13.64	11.51-17.16	9.57	14.56	43.2	1.77	12.96
Midrib length (cm)	12.6	10.48-15.96	9.57	14.71	42.32	1.62	12.82
Petiole length (cm)	1.04	0.83-1.52	14.65	21.54	46.3	0.21	20.54
Petiole appendages	2.07	1.29-2.53	12.96	17.97	52.03	0.4	19.26
Maximum leaf width (cm)	1.91	1.26-2.72	18.91	24.78	58.22	0.57	29.72
Number of teeth per cm on leaf margins	4.6	3.98-5.82	10.04	12.48	64.75	0.77	16.55

Maximum genetic advance value was recorded for total leaf length (1.77) and minimum value was recorded for petiole length (0.21). Genetic advance percentage of mean (genetic gain) value recorded maximum for maximum leaf width (29.72%) and minimum value was recorded for midrib length (12.82 %). The high heritability coupled with high genetic advance as percentage of mean was recorded for maximum leaf width which signifies the use of variability in advance breeding programs. The findings of the study was found contrary with the results of Sharma *et al.* (2017) as they found significant differences for leaf length, midrib length, leaf width and petiole length among the hybrids of willow in nursery. Sharma *et al.* (2019) estimates

the genetic parameters for leaf traits among willow clones and found high heritability with moderate genetic gain for number of leaf teeth which was comparable to our present study.

## CONCLUSION

The present study revealed that the variability estimates computed for each leaf trait exhibited significant variability among all the willow clones. This further suggested that the willow clones will be evaluated in the field and best clones will be selected for further tree improvement and breeding programs.



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# Incidence and Dispersion of Plant Parasitic Nematodes in Cauliflower Growing Regions of Tamil Nadu

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## ABSTRACT

A nematode survey was conducted in the Tamil Nadu districts of Dindigul, Theni, Krishnagiri, The Nilgiris and Coimbatore to investigate Plant Parasitic Nematodes (PPN) associated with cauliflower. The soil samples were analysed using a modified Baermann's funnel technique, and the root samples were analysed using an Acidfuchsin lactophenol solution to identify females. The survey results revealed that the root knot nematode (*Meloidogyne incognita*), lesion nematode (*Pratylenchus penetrans*), spiral nematode (*Helicotylenchus dihystera*), lance nematode (*Hoplolaimus indicus*), dagger nematode (*Xiphinema americanum*), and *Tylenchus filiformis* were associated with the cauliflower. The highest incidence of nematode damage was observed in Krishnagiri, followed by The Nilgiris and Coimbatore districts. The root knot nematode (*Meloidogyne incognita*) was found most frequently in all of the districts surveyed, with the highest incidences in Krishnagiri, The Nilgiris, and Coimbatore.

## HIGHLIGHTS

- The study revealed the occurrence of *Meloidogyne incognita*, *Helicotylenchus dihystera*, *Pratylenchus penetrans*, *Xiphinema americanum*, *Hoplolaimus indicus* and *Tylenchus filiformis* in Dindigul, Theni, Erode, The Nilgiris and Coimbatore districts of Tamil Nadu.
- The major genera associated with cabbage and cauliflower was *M. incognita* followed by *H. dihystera*, *P. penetrans*, *X. americanum* and *H. indicus*. Whereas *T. filiformis* was least found in all the districts surveyed.

**Keywords:** Nematode, survey, cauliflower, community analysis, *Meloidogyne incognita*

The term "cole crops" refers to various vegetables in the Cruciferous family. This family includes cauliflower, cauliflower, *knol-khol*, broccoli, Brussels sprouts, kale, and Chinese cauliflower. All crops in the Cruciferous family are grown as varieties of the species *Brassica oleracea*. Cole crops are widely grown all over the world, including in tropical, subtropical, and temperate climates. Cabbage (*Brassica oleracea* var. *capitata*) and cauliflower (*Brassica oleracea* var. *botrytis*) are considered major vegetables in India, while the rest of the vegetable crops in this family are considered minor vegetables. Cruciferous vegetables are high in vitamin A, and vitamin C contains significant amounts of minerals such as phosphorus, potassium, calcium, sodium, and iron (Fahey *et al.* 2001). According

to Strange and Scott (2005), plant parasitic pests and pathogens account for approximately 10% of global food production. Plant parasitic nematodes are estimated to cost the world \$ 125 billion each year. Root knot nematodes (*Meloidogyne* spp.), sting nematodes (*Belanolaimus* spp.), stubby-root nematodes (*Trichodorous* spp.), and cyst nematodes (*Heterodera* spp.) are plant parasitic nematodes associated with cruciferous vegetables that cause significant yield losses (Chitwood 2003). Root

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knot nematode (*Meloidogyne* spp.) has a positive correlation with cabbage diseases such as club root, damping off, and head rot (Loganthan 2002). Phytonematodes are the most diverse organisms, with varying distribution patterns, and they are also major pests of high-value agricultural crops. The pathogenic potential and population growth of nematodes determine the extent of damage. The community analysis of plant parasitic nematodes in various agricultural crops is a highly valuable factor, not only for assessing the pathogenic potential of the nematodes in a specific region, crop, or soil type, but also for identifying nematode infesting hotspots. However, no studies on the community analysis of plant parasitic nematodes associated with cabbage have been reported in Tamil Nadu. As a result, the study of nematode parasites associated with cauliflower in major growing regions may be considered the first documented in Tamil Nadu.

## MATERIALS AND METHODS

In the year 2018, a random survey was conducted for the community analysis of nematodes associated with cauliflower in major growing regions of Tamil Nadu. Diseased fields were identified based on crop aboveground symptoms such as wilting, stunted growth, and leaf yellowing. During the crop's midseason, soil and root samples were collected from the rhizosphere of cauliflower at a depth of 10-15 cm. A shovel was used to collect galled root samples from the soil (Sahu *et al.* 2011). Five plants were chosen at random from each locality, and three samples were collected from each plant. The soil samples were thoroughly mixed, and a composite sample of 250cc was collected in polythene bags labelled properly for analysis (Ilangovan *et al.* 2017). Root and soil samples were kept in a refrigerator at 4 degrees Celsius for no more than a week. Sixty soil and root samples were collected from cauliflower in five of Tamil Nadu's major cultivated districts. Decanting and wet sieving were used to extract nematodes from soil samples (Cobb NA, 1918). A modified Baermann's technique was used to collect nematode suspension (Schinder 1961). Root samples were homogenised with a warring blender, and migratory endoparasitic nematodes were separated using Baermann's technique. Endoparasitic nematodes were detected in infested root samples (galls) stained with acidfucshin

lactophenol (McBeth CB *et al.* 1941). Adult root knot females were excised from the stained roots, and perineal sections were prepared for species identification (Hartman and Sasser 1985). The nematodes were counted using a counting dish and a stereoscopic binocular microscope. A total nematode population involving soil (250cc) and root (5g) from major cauliflower growing regions was subjected to community analysis using Norton's formulae (1978);

Absolute frequency =

$$\frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100$$

Relative frequency =

$$\frac{\text{Frequency of a species}}{\text{Sum of frequencies of all species}} \times 100$$

Relative density =

$$\frac{\text{Number of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100$$

Absolute density =

$$\frac{\text{Number of individuals of a species in a sample}}{\text{Volume or mass or units of the sample}} \times 100$$

Prominence value =

$$\frac{\text{Absolute Density} \times \sqrt{\text{Absolute frequency}}}{100}$$

## RESULTS AND DISCUSSION

*Meloidogyne incognita*, *Helicotylenchus dihystra*, *Pratylenchus penetrans*, *Xiphinema americanum*, *Hoplolaimus indicus*, and *Tylenchus filiformis* were identified as six major genera of plant parasitic nematodes associated with cauliflower grown in the field. The species in various genera were identified using camera lucida diagrams and taxonomic keys. Female mature root knot nematodes were extracted at random from galled cauliflower roots. All the root knot nematodes having the posterior cuticular patterns showed the typical characters of high squarish dorsal arch, striae closely spaced, wavy to zigzag especially dorsally and laterally, lateral field

not clear. The second stage juveniles having the morphological characters of head without offset, truncate cone in lateral view, 2- 3 head annules and with prominent round spear knobs rounded. Males having the head with not offset. Truncate cone shape, clearly annulated, conus of spear longer than the shaft, spear knobs prominent and greater width than the length, concave, toothed or flat anterior regions, tail bluntly rounded and spicules slightly curved. Hence the nematode was identified as *Meloidogyne incognita* based on posterior cuticular pattern and head and tail regions of male and second stage juveniles. Among the plant parasitic nematodes associated with cauliflower, the root knot nematode *M. incognita* was considered as the most predominant followed by *H.dihystera*, *P. penetrans*, *X. americanum*, *H. indicus* and *T. filiformis* based on their total population recorded district wise. The population of *M. incognita* was considered most predominant nematode followed by *H. dihystrera*, *P. pentrans*, *X. americanum*, *H. indicus* and *T. filiformis* based on their total population recorded in different districts. The population of *M. incognita*, *H. dihystrera*, *P. pentrans*, *T. filiformis*, *X. americanum* and *H. indicus* were ranged from 73.77 to 281.00, 34.15 to 104.00, 10.31 to 25.29, 1.33 to 15.63, 13.88 to 37.33 and 2.40 to 5.00. (Table 1: Fig. 1).

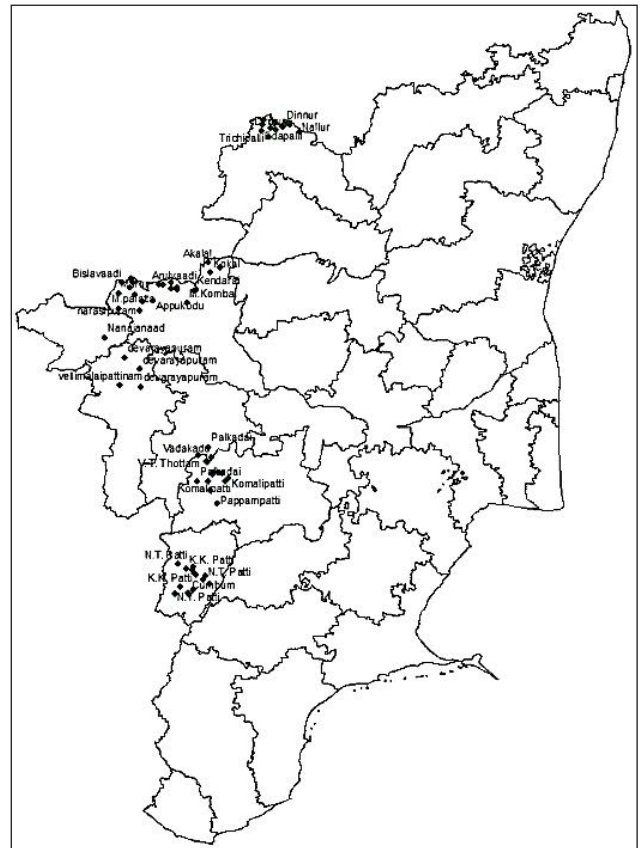


Fig. 1: Areas sampled for plant-parasitic nematodes associated with cauliflower growing regions of Tamil Nadu. (QGIS SOFTWARE, 2016)

Table 1: Nematode population in cauliflower growing regions of Tamil Nadu (250cc soil + 5g root)

	<i>M. i</i>	<i>H. d</i>	<i>P. p</i>	<i>T. f</i>	<i>X. a</i>	<i>H. i</i>
<b>Theni District</b>						
Cumbum Block	146.80	46.20	21.40	6.60	23.00	1.60
Chinnamanur Block	72.93	52.40	17.80	10.20	15.00	2.20
<b>Erode District</b>						
Thalavadi Block	91.38	80.50	9.88	3.38	14.75	5.88
<b>Nilgiris District</b>						
Udagamandalam Block	222.67	23.00	12.50	2.50	17.75	8.25
<b>Krishnagiri district</b>						
Hosur block	262.48	76.38	14.00	10.88	19.63	1.38
<b>Coimbatore</b>						
Thondamuthur block	237.00	59.43	20.86	1.29	26.29	2.86
Total	1033.26	337.90	96.43	34.84	116.41	22.16

\*Values in columns are mean of sixty samples (250cc soil + 5g root)

*M. i* – *Meloidogyne incognita*, *H.d*- *Helicotylenchus dihystrera*, *P. p*- *Pratylenchus penetrans*, *T. f* – *Tylenchus filiformis*, *X. a*- *Xiphinema americanum*, *H. i*- *Hoplolaimus indicus*.

## Occurrence and community structure of nematodes in cauliflower

### Absolute frequency

The root knot nematode, *M. incognita* was most frequently occurred in Hosur block of Krishnagiri district with absolute frequency of 76.1 % and least frequently occurred in Oddanchathiram block of Dindigul district having absolute frequency value 28.57 % among the districts surveyed. The spiral nematode, *H. dihystra* was most frequently occurred in Oddanchathiram block with absolute frequency of 85%. Chinnamanur block of Theni district with least absolute frequency of 28.5%. The lesion nematode, *P. penetrans* was most frequently occurred in Oddanchathiram block of Dindigul district with absolute frequency 71.4 % and least frequently (21.4%) occurred in Chinnamanur block. *T. filiformis* was most frequently occurred in Oddanchathiram block (42.8 %) and least frequency (4.7%) occurred in Hosur block of Krishnagiri district (Table 3a). The dagger nematode, *X. americanum* was most frequently occurred in Oddanchathiram block with highest absolute frequency (57.1 %) and least frequently occurred in Thondamuthur (21.0 %) block of Coimbatore district. The lance nematode *H. indicus* was most frequently occurred in Oddanchathiram block of Dindigul district with the absolute frequency of 28.5 % and least frequency was recorded in Palani block of Dindigul district and Cumbum block of Theni district (7.6 %) (Table 3a).

### Relative frequency

The relative frequency of *M. incognita* was highest in Hosur block (37.2%) followed by Chinnamanur block (34.4%). The least relative frequency value was found in Oddanchathiram (9.0%) block. The relative frequency (30.5%) of *H. dihystra* was found higher in Thondamuthur block of Coimbatore district followed by Hosur of Krishnagiri district (30.2%). The least relative frequency (17.1%) was found in Udhamandalam block of The Nilgiris district. The relative frequency of *P. penetrans* was highest (22.7%) in Oddanchathiram, Dindigul district followed Thondamuthur (22.2%). Least relative frequency (11.6%) was found in Hosur of Krishnagiri district. The relative frequency of *T. filiformis* was highest in Oddanchathiram block (13.6%) followed by Palani block (11.1%). The least relative frequency (2.3%) was found in Hosur block of Krishnagiri district. The relative frequency of *X. americanum* was found highest (21.7%) in Chinnamanur block. The lowest relative frequency (11.1%) was in Thondamuthur block (Table 2). The relative frequency of *H. indicus* was found highest (9.0%) in Oddanchathiram block. The lowest relative frequency (3.7%) of this genus was in Palani block (Table 3b).

### Absolute density

The absolute density of *M. incognita* was highest (112.4%) in Hosur block of Krishnagiri district followed by Thondamuthur, Coimbatore district (100.6%). The least absolute density (52.2%) recorded

**Table 2:** Prominence value of nematodes associated with cauliflower

	<i>M. i</i>	<i>H. d</i>	<i>P. p</i>	<i>T. f</i>	<i>X. a</i>	<i>H. i</i>
<b>Dindigul District</b>						
Palani Block	3.66	2.42	0.38	0.30	0.47	0.03
Oddanchathiram Block	3.03	2.94	0.85	0.24	0.76	0.08
<b>Theni District</b>						
Cumbum Block	4.10	2.09	0.34	0.17	0.31	0.03
Chinnamanur Block	5.25	0.73	0.19	0.05	0.35	0.06
<b>Nilgiris District</b>						
Udagamandalam Block	8.25	1.42	0.43	0.09	0.47	0.04
<b>Krishnagiri district</b>						
Hosur block	9.81	3.27	0.33	0.05	0.47	0.03
<b>Coimbatore</b>						
Thondamuthur Block	7.30	1.26	0.45	0.01	0.69	0.06

*M. i* – *Meloidogyne incognita*, *H. d*- *Helicotylenchus dihystra*, *P. p*- *Pratylenchus penetrans*, *T. f* – *Tylenchus filiformis*, *X. a*- *Xiphinema americanum*, *H. i*- *Hoplolaimus indicus*.



**Table 3a:** Community analysis of nematodes in different districts of Tamil Nadu on Cauliflower

Blocks	Absolute Frequency (%)						Relative Frequency (%)					
	<i>M. i</i>	<i>H. d</i>	<i>P. p</i>	<i>T. f</i>	<i>X. a</i>	<i>H. i</i>	<i>M. i</i>	<i>H. d</i>	<i>P. p</i>	<i>T. f</i>	<i>X. a</i>	<i>H. i</i>
<b>Dindigul District</b>												
Palani Block	46.15	61.54	30.77	23.08	38.46	7.69	22.22	29.63	14.81	11.11	18.52	3.70
Oddanchathiram Block	28.57	85.71	71.43	42.86	57.14	28.57	9.09	27.27	22.73	13.64	18.18	9.09
<b>Theni District</b>												
Cumbum Block	61.54	46.15	23.08	15.38	30.77	7.69	33.33	25.00	12.50	8.33	16.67	4.17
Chinnamanur Block	57.14	28.57	21.43	7.14	35.71	14.29	34.78	17.39	13.04	4.35	21.74	8.70
<b>Nilgiris District</b>												
Udagamandalam Block	68.75	37.50	43.75	18.75	37.50	12.50	31.43	17.14	20.00	8.57	17.14	5.71
<b>Krishnagiri District</b>												
Hosur block	76.19	61.90	23.81	4.76	28.57	9.52	37.21	30.23	11.63	2.33	13.95	4.65
<b>Coimbatore District</b>												
Thondamuthur block	52.63	57.89	42.11	5.26	21.05	10.53	27.78	30.56	22.22	2.78	11.11	5.56

*M. i* – *Meloidogyne incognita*, *H. d*– *Helicotylenchus dihystera*, *P. p*– *Pratylenchus penetrans*, *T. f* – *Tylenchus filiformis*, *X. a*– *Xiphinema americanum*, *H. i*– *Hoplolaimus indicus*.

**Table 3b:** Community analysis of nematodes in different districts of Tamil Nadu on cauliflower (250cc soil + 5g root)

Blocks	Absolute Density (%)						Relative Density (%)					
	<i>M. i</i>	<i>H. d</i>	<i>P. p</i>	<i>T. f</i>	<i>X. a</i>	<i>H. i</i>	<i>M. i</i>	<i>H. d</i>	<i>P. p</i>	<i>T. f</i>	<i>X. a</i>	<i>H. i</i>
<b>Dindigul District</b>												
Palani Block	53.82	30.90	6.90	6.25	7.50	1.25	9.88	16.41	14.25	29.45	12.44	12.86
Oddanchathiram Block	56.69	31.71	10.11	3.71	10.06	1.43	10.40	16.84	20.89	17.50	16.68	14.69
<b>Theni District</b>												
Cumbum Block	52.25	30.75	7.15	4.35	5.55	1.10	9.59	16.33	14.77	20.49	9.21	11.31
Chinnamanur Block	69.51	13.66	4.13	1.99	5.86	1.72	12.76	7.25	8.52	9.38	9.72	17.66
<b>Nilgiris District</b>												
Udagamandalam Block	99.47	23.13	6.47	2.07	7.67	1.27	18.26	12.29	13.36	9.74	12.72	13.03
<b>Krishnagiri District</b>												
Hosur block	112.40	41.60	6.72	2.32	8.72	0.96	20.63	22.09	13.88	10.93	14.46	9.87
<b>Coimbatore District</b>												
Thondamuthur Block	100.67	16.53	6.93	0.53	14.93	2.00	18.48	8.78	14.32	2.51	24.77	20.57

*M. i* – *Meloidogyne incognita*, *H. d*– *Helicotylenchus dihystera*, *P. p*– *Pratylenchus penetrans*, *T. f* – *Tylenchus filiformis*, *X. a*– *Xiphinema americanum*, *H. i*– *Hoplolaimus indicus*.

in Chinnamnur, Theni district. The absolute density of *H. dihystera* was highest (41.6%) in Hosur of Krishnagiri district followed by Oddanchathiram, Dindigul district (31.7%). The lowest value (13.6%) was found in Chinnamanur, Theni. The absolute density of *P. penetrans* was highest (10.1%) in Oddanchathiram, Dindigul district followed by Cumbum, Theni district (7.1%) The least value (4.1%) was found in Chinnamanur, Theni district (Table 3b). The absolute density of *T. filiformis* was highest (6.2%) in Palani, Dindigul district followed

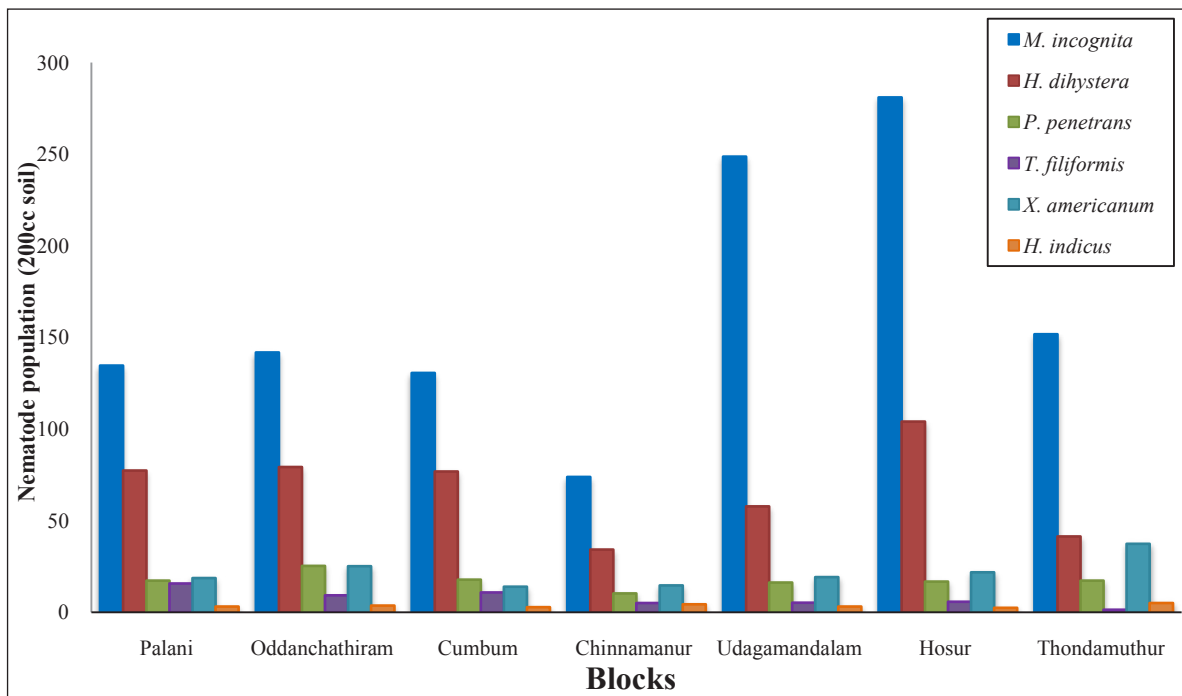
by Cumbum, Theni district (4.3%) The least value (0.5%) was found in Thondamuthur, Coimbatore district. The absolute density of *X. americanum* was highest (14.9%) in Thondamuthur, Coimbatore district followed by Oddanchathiram, Dindigul district (10%). The lowest value (5.5%) was recorded from Cumbum, Theni district. The absolute density of *H. indicus* was highest (2%) in Thondamuthur, Coimbatore district. Lowest absolute density (0.9%) was recorded in Hosur, Krishnagiri district (Table 3b).

## Relative density

Relative density of *M. incognita* was highest (20.6%) in Hosur block of Krishnagiri district followed by Thondamuthur, Coimbatore district (18.4%). The lowest value (9.5%) was recorded from Cumbum, Theni district. Relative density of *H. dihystra* was found to be highest (22.0%) in Hosur block of Krishnagiri district followed by Oddanchathiram, Dindigul district (16.8%). Least value (7.2%) was recorded in Chinnamanur of the Theni district. Relative density of *P. penetrans* was found to be highest (20.8%) in Oddanchathiram, Palani district followed by Thondamuthur, Coimbatore district (14.3%). Least value (8.5%) was recorded in Chinnamanur block of Erode district. Relative density of *T. filiformis* was found highest (29.4%) in Palani, Dindigul district followed by Cumbum, Theni (20.4%). Least value (2.5%) was recorded in Thondamuthur block of Coimbatore district. Relative density of *X. americanum* was found to be highest (24.7%) in Thondamuthur followed by Oddanchathiram, Palani district (16.6%). Least value (9.2%) was recorded from Cumbum, Theni district. Relative density of *H. lindicus* in was highest (20.5%) in Thondamuthur block of Coimbatore district. Least value (9.8%) was recorded from Hosur block of Krishnagiri district (Table 3b).

## Prominence value

The prominence value of *M. incognita* was highest (9.8) in Hosur block of Krishnagiri district. Least value (3.0) was recorded from Oddanchathiram block of Dindigul district. The prominence value (3.2) of *H. dihystra* was highest in Hosur, Krishnagiri district. Lowest value (0.7) was recorded from Chinnamanur block of Theni district. The prominence value of *P. penetrans* was highest (0.8) in Oddanchathiram block of Dindigul district. Lowest value (0.1) was recorded from Chinnamanur block of Theni district (Table 2). The prominence value of *T. filiformis* was highest (0.3) in Palani block of Dindigul district. Lowest value (0.01) was recorded from Thondamuthur block of Coimbatore district (Table 2; Fig. 3). The prominence value of *X. americanum* was found highest (0.7) in Oddanchathiram block of Dindigul district followed by Hosur block of Krishnagiri, Palani block of Dindigul and Uthagamandalam block of The Nigiris districts (0.4). Lowest value (0.3) was recorded from Cumbum, Theni district. The prominence value of *H. indicus* was highest (0.08) in Oddanchathiram block of Dindigul followed by Chinnamanur, Theni district and Thondamuthur block of Coimbatore district (0.06). Lowest value (0.03) was recorded from Cumbum of Theni district



**Fig. 2:** Nematode population in cauliflower growing regions of Tamil Nadu (250cc soil + 5 g root)

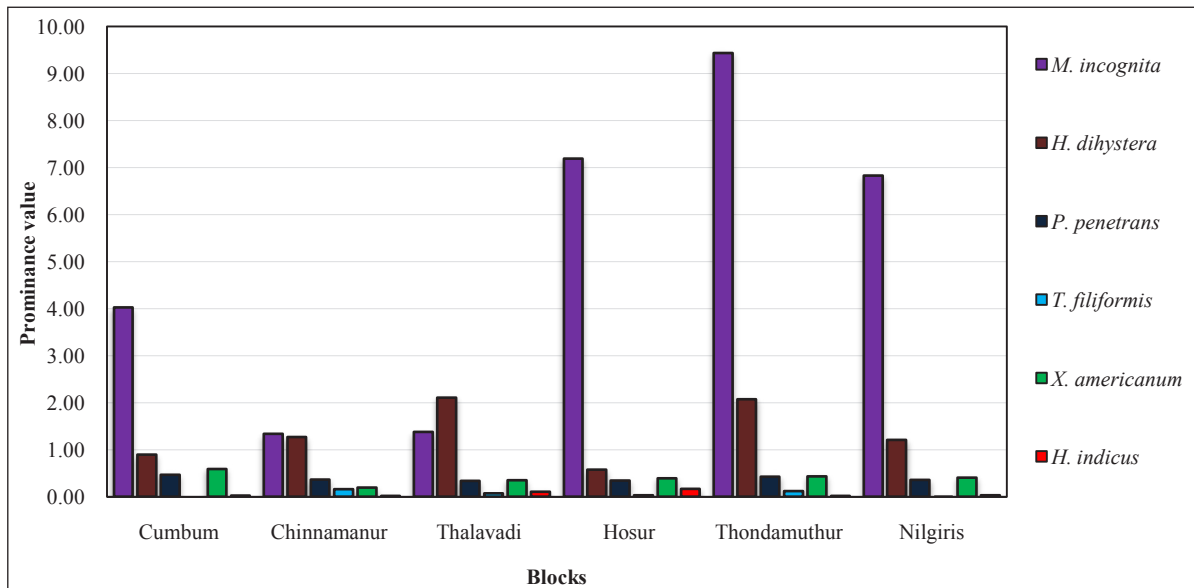


Fig. 3: Prominence value of nematodes associated with cauliflower

and Hosur block of Krishnagiri district (Table 2; Fig. 3).

It is confirmed that the documentation of research studies made by several authors, *M. incognita*, *H. dihystra*, *P. penetrans* and *T. filiformis* were the key nematode pests of cauliflower (Loganathan *et al.* 2010; Sahu *et al.* 2011; Waceke *et al.* 2014).

Based on the nematode densities with their total population, nematode infested hotspots were identified by calculating the exceeded damage threshold level ( $>$  single  $J_2$  / g of soil). The study revealed that Hosur block was the hot-spots of *M. incognita* in cauliflower with the nematode population of 262.48 per 250 cc soil and 5 g root sample (Table 1: Fig. 1). Thondamuthur (237.00) and Udhamandalam (222.67) blocks having the nematode populations nearer to the damage threshold level (Table 1: Fig. 1). The nematode infested hotspot noted in the current scenario proved that the cauliflower cultivation in Hosur block is certainly infested with plant parasitic nematodes.

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# Assessment of the Impact of Genetically Modified Cotton (*Bt* Cotton) on Soil Microbial Ecosystem

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## ABSTRACT

*Bt* cotton which confers resistance to important insect pests of cotton, occupy around 86% of total cotton area in India. Since crystal protein gene is expressed constitutively in *Bt* cotton have, crystal protein (Cry toxin) is produced in all over the plant parts and its complete life cycle. So, it may be considered that GM plants (*Bt* cotton) has potential to significantly change the functions such as nutrient mineralization, carbon turnover and plant growth through the products of introduced genes or modified rhizosphere chemistry. This study was conducted to observe the effect of *Bt* cotton (presence of Cry protein in root exudates) on efficiency of functional microbes such as *Trichoderma viride* and *Pseudomonas fluorescens* present in the soil rhizosphere and soil enzymes activities (dehydrogenase and urease) at different growth stages. Results of *Trichoderma viride* and *Pseudomonas fluorescens* isolated from *Bt* and non *Bt* cotton rhizosphere antagonistic study by dual culture method revealed that some variation was observed in the pathogen inhibition percentage between isolates obtained from Non *Bt* and *Bt* cotton rhizosphere. Some of the isolates obtained from *Bt* rhizosphere showed better inhibition than its counterpart. However, the efficiency did not significantly vary between *Bt* and Non *Bt* isolates indicating that *Bt* protein does not have any impact on microbes functional activity. Regarding of enzymes activity dehydrogenase and urease enzymes activity were high in *Bt* cotton rhizosphere as compared to non *Bt* rhizosphere. Statistically urease enzymes was not significantly differed between *Bt* and non *Bt* cotton, but dehydrogenase activity was significantly high ( $P < 0.05$ ) in the *Bt* cotton rhizosphere as compared to non *Bt* cotton rhizosphere.

## HIGHLIGHTS

- Antagonistic activity of *Trichoderma viride* and *Pseudomonas fluorescens* isolates obtained from *Bt* and non *Bt* cotton rhizosphere against *Rhizoctonia solani* is not differed significantly.
- No significant differences observed in urease activity between *Bt* and non *Bt* cotton rhizosphere.
- Dehydrogenase enzyme activity was more in *Bt* cotton rhizosphere compared to non *Bt* cotton rhizosphere.

**Keywords:** *Bt* cotton, Crystal protein, Root exudates, Functional microbes, Antagonistic activity and Enzymes activity

A unique feature of *Bt* cotton is production of crystal-like proteins all over the plants with whole crop period by the expression of *cry* genes that selectively kill the specific groups of insects (Hardee *et al.* 2001). All *Bt* cotton plants contain a one or more foreign genes derived from the *Bacillus thuringiensis* (*Bt*) *i.e.*, Bollgard I with *cry1ac* and Bollgard II with *cry1ac* and *cry2ab* genes. The exudates of *Bt* cotton

plants are found to be rich in endotoxin *i.e.* Cry protein (Shen *et al.* 2005). The potential risks of genetically modified plants to environmental and

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human health have been of concern in recent years, due to the release of transgenic crop plants worldwide and their replacement of traditional crops (Nap *et al.* 2003). Many of the risk assessments have focused on the effects of transgenes on the soil ecosystem.

Some studies indicate that *Bt* cotton has no negative effects on soil flora and fauna and may even have beneficial effects (Saxena and Stotzky 2001), while some have reported adverse effects (Cui and Xia 2000; Tan *et al.* 2002). In the last decade many reports on potential impacts of transgenic crops on the structure and functioning of the soil microbial community have been published. Two of three transgenic *Bt* cotton lines caused a transient increase in total bacterial and fungal population levels; in contrast, neither the third transgenic *Bt* cotton line nor the purified *Bt* toxins affected the total numbers of bacteria and fungi (Donegan *et al.* 1995).

Biocontrol of soil-borne plant pathogens affecting agricultural plants can be controlled by the use of species of *Trichoderma* i.e., *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma hamatum*, and some antagonistic bacteria like *Pseudomonas fluorescens*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Streptomyces* spp. (Chet 1990). *Rhizoctonia solani* is a plant pathogenic fungus with a wide host range and worldwide distribution. It is one cause of the condition known as damping off resulting in the death of seedlings. The use of these antagonists for suppression of *R. solani* has been demonstrated by several workers (Ali and Nadarajah 2013; Rini and Sulochana 2006 and Bautista *et al.* 2007).

Enzymes are the vital activators in life processes, likewise in the soil they are known to play a substantial role in maintaining soil health and its environment. The enzymatic activity in the soil is mainly of microbial origin, being derived from intracellular, cell associated or free enzymes. These include dehydrogenase, urease, phosphatases, nitrate reductase, cellulases, and phenol oxidases (Tabatabai 1994 a,b). The biological activity in soil is largely concentrated in the top soil, the depth of which may vary from a few to 30 cm. These biological components consist mainly of soil organisms, especially microorganisms. Transgenic plants have the potential to significantly change the microbial dynamics and essential ecosystem functions such as nutrient mineralization, disease incidence, carbon turnover and plant growth

through the products of introduced genes or modified rhizosphere chemistry or altered crop residue quality (Gupta and Watson 2004). A decrease in specific microbial populations would lead to a decrease in decomposition processes and have secondary effects on plant pathogen survival and build up, as well as soil organic matter level and composition (Termorshuizen and Lotz 2002).

Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors (Kandeler 1996). Urease enzyme is responsible for the hydrolysis of urea fertilizers applied to the soil into  $\text{NH}_3$  and  $\text{CO}_2$  with the concomitant rise in soil pH (Andrews *et al.* 1989 ; Byrnes and Amberger 1989). Soil samples from the rhizosphere of *Bt* and non *Bt* cotton plants were collected at regular interval (30 days interval) coinciding with different growth stage of cotton analysed for dehydrogenase, and urease enzymes activities. On average dehydrogenase, and urease enzymes activity was high in *Bt* cotton rhizosphere as compared to non *Bt* rhizosphere. Except dehydrogenase enzyme, differences in activity of alkaline phosphatase, nitrate reductase and urease enzymes between *Bt* and non *Bt* plants rhizosphere were statistically non significant (Mina *et al.* 2011).

Enzyme activities in rhizosphere soil samples obtained after vegetative, reproductive, and senescing stages of growth and after a complete life cycle of cotton growth showed no significant differences between transgenic *Bt* and non-*Bt* cotton (Shen *et al.* 2005).

The rhizosphere (the zone directly surrounding and influenced by plant roots) contains a large majority of the soil's biota populations (>10-fold of that in the bulk soil) and the plant-microbe interaction in the rhizosphere is one of the major factors regulating the health and growth of plants. It is also widely acknowledged that root exudates govern the organisms in the rhizosphere (Lynch 1994; Bardgett *et al.* 1999). The exudates of *Bt* plants are found to be rich in endotoxin i.e., Cry protein.

An evaluation of the ecological risks of *Bt* cotton was made on the basis of changes in a number of biological and biochemical properties of soil. Though scientific information on the effect of *Bt* protein on the functional microbes is available but it is not specific to the particular group of beneficial

microbes. Thus, the aim of the experiment was to determine the effect of *Bt* cotton (RCH-2 BG II) on soil enzymes like dehydrogenases and urease and assess the impact of *Bt* protein on functional activity of beneficial rhizosphere inhabiting microorganisms such as *Trichoderma viride* and *Pseudomonas fluorescens* and its antagonistic effect against *R. solani* in cotton.

## MATERIALS AND METHODS

### Preparation of inoculums and seed treatment for Pot culture study

The cultures of *Trichoderma viride* and *Pseudomonas fluorescens* (biocontrol agents) for cotton and *R. solani* (plant pathogenic fungi) were obtained from Plant Pathology department, TNAU for the present research. *Trichoderma viride* discs were transferred to Potato Dextrose Broth and kept in room temperature for 4 Days. *Pseudomonas fluorescens* was inoculated in KB broth, kept for incubation on incubator cum shaker for 72 hours at 32 °C. After three days the *T. viride* mat formed on the surface of the medium was homogenized and these microbial cultures were used to treat the RCH-2 (Non-*Bt*) and RCH-2 BG II (*Bt* cotton) seeds. Seeds were mixed with microbial culture containing 10<sup>8</sup> CFU/ml culture. *Bt* and non *Bt* cotton seeds were obtained from Rasi Seeds Pvt. Ltd., Attur, Tamil Nadu. Cotton seeds were surface sterilized for 2 min with 70% ethanol followed by 2% sodium hypochlorite (10 min) and rinsed in sterile distilled water. A Quantity of 10 ml of culture was mixed with 0.2 g of CMC (2 %) to treat 5 gram each *Bt* and Non-*Bt* cotton seeds. Seeds were mixed with culture inoculums and kept for incubation for half an hour. After incubation treated seeds were shade dried for half an hour before sowing in pots.

### Biocontrol agent with *Rhizoctonia solani*

Completely Randomized Design (CRD) was adopted for pot culture study. Total 6 treatments (Table 1) were chosen for the pot culture with four replication of each treatment. *Bt* and Non *Bt* cotton seeds treated with *Trichoderma viride* and *Pseudomonas fluorescens* was sown in the presence of plant pathogen *Rhizoctonia solani* (causal agent for root rot in cotton). The plastic pots (12 cm dia.) filled with sterilized clay loam soil were inoculated with one-week-old culture of *R. solani*, prepared on corn meal sand medium as described by Mathew

and Gupta (1998) @ 2g/kg soil and allowed to stabilize for 5 days, then surface sterilized seeds were sown @ 2 seeds per pot. *Trichoderma viride* and *P. fluorescens* were isolated from *Bt* and Non *Bt* cotton rhizosphere at 15 Days interval i.e., 15, 30 and 45 DAS.

### Analysis of *Trichoderma viride* and *P. fluorescens* antagonistic activity

All the *Trichoderma viride* and *P. fluorescens* isolates obtained at 15 days intervals were evaluated for their antagonistic activity against the pathogens under *in vitro* conditions. The assay for antagonism was performed on Potato Dextrose Agar (PDA) adopting dual culture method (Dennis and Webster, 1971). A 5 mm diameter disc of *Trichoderma viride* was placed individually at one end of the Petri plate containing PDA and just opposite end to that a 5 mm diameter disc of the pathogen (*R. solani*) was placed. In control, only pathogen was inoculated and incubated at 28± 2° C temperatures in BOD incubator. Each treatment was replicated thrice. Inhibition of *R. solani* growth was recorded after 72 h.

A 5 mm of mycelia agar disc from test fungal pathogen cultures was placed on the one side of a Petri plate containing PDA: KB (1:1, v/v) media. The plates were then incubated at 25 °C for 24 hrs. A loopful of test antagonistic bacterial culture was streaked on the opposite side of disc of pathogen on the same dish. PDA: KB (1:1, v/v) media plates inoculated only with pathogen were maintained as control. The zone of inhibition was recorded as the distance between the fungal pathogen and the area of antagonist growth after 72 h. The percentage inhibition of the growth of pathogen was calculated with the help of the formula given by Whipps (1997). This experiment was conducted in completely randomized design with three replications and the data were subjected to analysis of variance.

$$PI = \frac{C - T \times 100}{C}$$

Where,

PI = Percent inhibition of mycelial growth

C = Radial growth of pathogen in control plates (mm)

T = Radial growth of pathogen in dual culture (mm)



## Soil Sampling for enzyme study

*Bt* and non *Bt* cotton plants were planted in the field and Soil samples from the rhizosphere of *Bt* cotton and non *Bt* cotton plants were collected at regular interval (30 days interval, coinciding with different growth stage of cotton) till harvesting. First Sampling was done, 30 Days After Sowing (DAS) (at seedling stage, growth stage 1), second sampling 60 DAS (at vegetative stage, growth stage 2), third sampling 90 DAS (at Flowering stage growth stage 3), fourth sampling 120 DAS (at Boll formation stage, growth stage 4) and last fifth sampling 150 DAS (at mature stage, growth stage 5). Five rhizosphere samples (0-15cm) were taken in an each plot and were mixed to make a representative sample for analysis. Collected rhizospheric soil samples were air dried, ground and sieved (passed through a 1 mm sieve) and analysed for all enzymes activities except dehydrogenase. For observing dehydrogenase the soil samples were kept moist.

### Estimation of dehydrogenase activity

Dehydrogenase activity was analyzed as described by Min *et al.* (2001). Five grams of soil was incubated for 12 h at 37 °C in 5 ml of a TTC solution (5 g TTC in 0.2M Tris-HCl buffer, pH 7.4). Two drops of concentrated sulfuric acid were added immediately after the incubation to end the reaction. The sample was then blended with 5 ml of toluene and shaken for 30 min at 250 rpm, followed by centrifuging at 4500 g for 5 min to extract TPF. The optical density of the red colour extract supernatant was measured at 492 nm using UV-Vis spectrophotometer (UV-1201, Shimadzu Corp, Japan). Soil dehydrogenase activity was expressed as  $\mu\text{g TPF g}^{-1} 12 \text{ h}^{-1}$ .

### Estimation of urease activity

Urease enzyme activity in soil sample was estimated according to "determination of urea remaining" methodology (Tabataba 1994b). This methodology estimate urea hydrolysis in soils on account of urease enzyme activity. Five gram of soil was mixed with 5 ml of urea solution (10 mg urea/ml) in a 50-ml erlenmeyer flask, and incubated for 5 h at 37 °C. After 5 h, 50 ml 2M KCl-PMA was added to flasks and kept for 1h shaking. After 1 h shaking soil suspension was filtered under suction through Whatmann no 42 filter paper. Out of filtrate 2 ml of aliquot was taken and

mixed with 10 ml 2M KCl-PMA and 30 ml colouring reagent (25 ml 2.5% DAM + 10 ml of 0.25% TSC in 500 ml acid reagent). This mixture was first kept in water bath for 30 minutes and then kept in ice coldwater for 15 minutes for colour development. Absorbance of coloured end product was measured at 527 nm. Activity of Urease enzyme in soil was expressed as  $\mu\text{g urea N hydrolysed g}^{-1} \text{ h}^{-1}$ .

## RESULTS

Inhibition percentage of *T. viride* and *P. fluorescens* isolated from different stages (15 DAS interval) of Non *Bt* and *Bt* cotton rhizosphere were tested for their efficacy against *Rhizoctonia solani* by dual culture method (Fig. 1). The details of inhibition percentage are given in Table 2. *T. viride* isolated from *Bt* cotton rhizosphere at 15 DAS recorded the highest inhibition percent (62.8%) and where as *T. viride* obtained from Non *Bt* cotton inhibition percent (61.1%) was slightly lesser then *Bt* cotton isolate . *P. fluorescens* registered the highest inhibition percent (51.6%) and (50.4%) isolated from Non *Bt* and *Bt* cotton rhizosphere at 45 DAS respectively. Inhibition percentage was high in *Bt* cotton *T. viride* isolates in all sampling times then non *Bt* cotton isolates. In case of *P. fluorescens* inhibition pattern was differed only 15 DAS *Bt* cotton rhizosphere isolates was superior then Non *Bt* cotton rhizosphere remaining 30 and 45 DAS isolates were *vice versa*. In *in vitro* (lab) condition inhibition pattern was varied between isolates recovered from *Bt* and Non *Bt* cotton rhizosphere but the of the variation observed was not Statistically significant.

**Table 1:** Details of different treatments used for pot culture

Sl. No.	Treatments
1	Cotton (Control)
2	Cotton + <i>Trichoderma viride</i>
3	Cotton + <i>Pseudomonas fluorescens</i>
4	<i>Bt</i> Cotton (Control)
5	<i>Bt</i> Cotton + <i>Trichoderma viride</i>
6	<i>Bt</i> Cotton + <i>P. fluorescens</i>

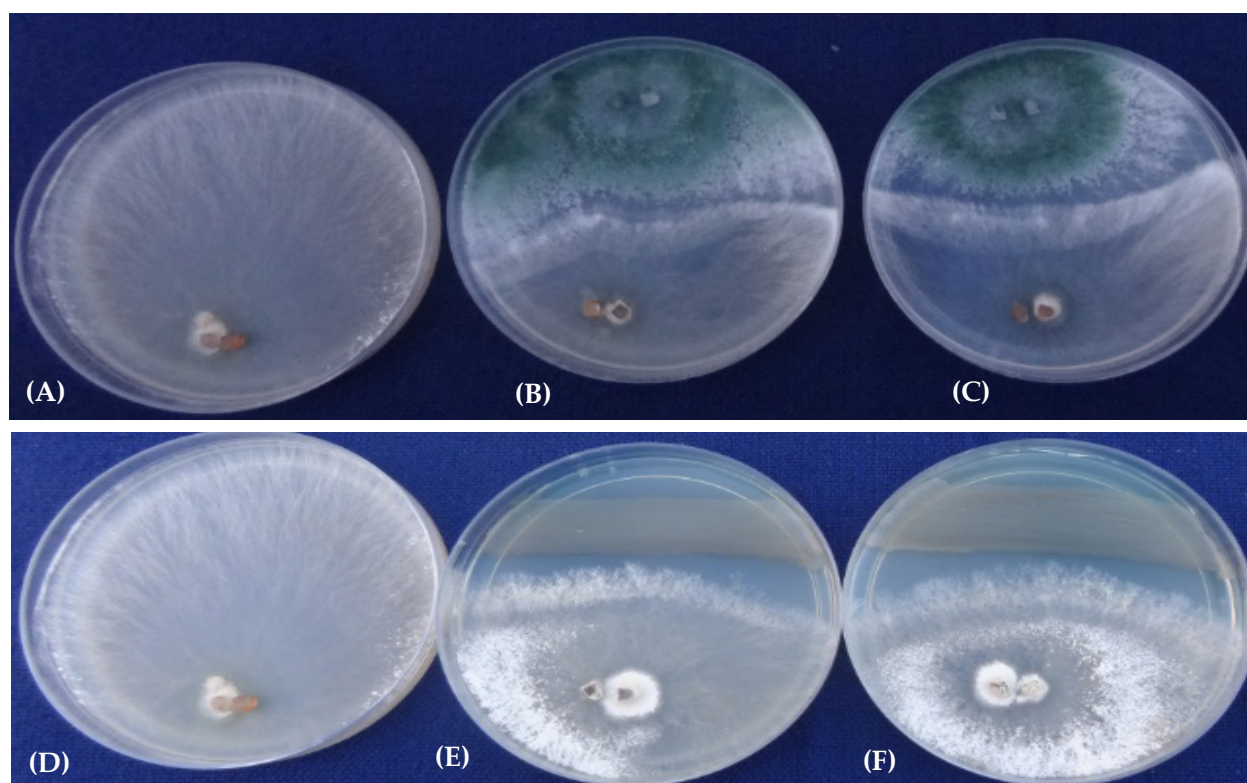
### Soil functional enzyme study

Soil samples collected from the rhizosphere of *Bt* cotton and non *Bt* cotton plants at five different growth stage of cotton were studied to know the impact of *Bt* cotton on soil enzymes.



**Table 2:** *In-vitro* evaluation of recovered biocontrol agents from *Bt* and non *Bt* rhizosphere soil against *Rhizoctonia solani*

Different days	Mycelial growth (mm) Mean radial growth (mm) of three replications				Mycelial growth inhibition (%)			
	<i>T. viride</i>		<i>P. fluorescens</i>		<i>T. viride</i>		<i>P. fluorescens</i>	
	Cotton	<i>Bt</i> Cotton	Cotton	<i>Bt</i> Cotton	Cotton	<i>Bt</i> Cotton	Cotton	<i>Bt</i> Cotton
15	35.0	33.4	45.5	45.2	61.1	62.8	49.4	50.0
30	36.0	35.0	44.1	45.3	60.0	61.1	51.0	49.6
45	36.7	36.0	43.6	44.6	59.2	60.0	51.6	50.4
Mean	35.9	34.8	44.4	45.0	60.1	61.3	50.6	50.0
	<b>D</b>	<b>O</b>	<b>C</b>	<b>D × O</b>	<b>O × C</b>	<b>D × C</b>	<b>D × O × C</b>	
S.Ed	0.58	0.48	0.48	0.83	0.68	0.83	1.17	
CD	NS	0.99	NS	1.71	NS	NS	NS	

A, D – Control B, E – Isolates from *Bt* cotton C, F – Isolates from Non *Bt* cotton**Fig. 1:** Antagonistic activity of *T. viride* and *P. fluorescens* isolated from 30 DAS cotton rhizosphere against *R. solani* on dual culture agar plate

### Dehydrogenase activity

Dehydrogenase activity was highest in the *Bt* (13.1  $\mu\text{g}$  of TPF  $\text{g}^{-1}$  dry soil in  $24^{-1}$ ) at maturity stage. However non-*Bt* cotton recorded the highest activity (11.2  $\mu\text{g}$  of TPF  $\text{g}^{-1}$  dry soil in  $24^{-1}$ ) at boll formation stage. Enzyme activity varied significantly between the five sampling times (8.5 to 13.1 and 7.6 to 11.2  $\mu\text{g}$  of TPF  $\text{g}^{-1}$  dry soil in  $24^{-1}$  *Bt* and non *Bt* cotton respectively). Activity of the enzyme increased at later crop

growth stage. On an average dehydrogenase enzyme activity was high in *Bt* cotton rhizosphere as compared to non *Bt* rhizosphere. The enzyme activity was high at Boll forming stage in Non *Bt* cotton where as remaining all the stages *Bt* cotton rhizosphere had higher activity. Effect of cultivars, growth stages and interaction between cultivars, growth stages on the dehydrogenase activity of the *Bt* and non *Bt* cotton rhizosphere soil sample were significant (Table 3).

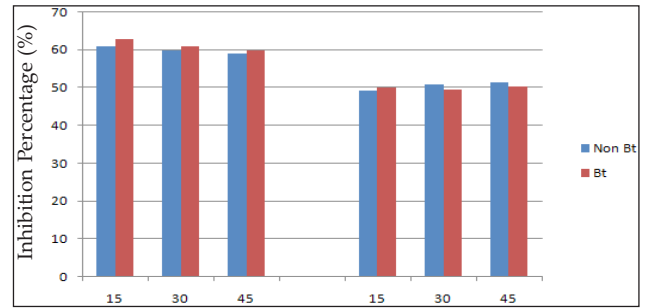
### Urease activity

Urease activity was high in the *Bt* cotton (138.7 µg Urea N hydrolyzed g<sup>-1</sup> dry soil hour<sup>-1</sup>) at vegetative stage followed by non- *Bt* cotton (135.2 µg Urea N hydrolyzed g<sup>-1</sup> dry soil hour<sup>-1</sup>) at seedling stage. Activity of the enzyme was high in early stages i.e., seedling and vegetative stage then decreased in remaining crop growth stages. Enzyme activity was found high in vegetative, boll forming and maturity stage in *Bt* cotton rhizosphere. On an average urease enzyme activity was slightly high (129.94 µg Urea N hydrolyzed g<sup>-1</sup> dry soil hour<sup>-1</sup>) in *Bt* cotton rhizosphere as compared to non *Bt* rhizosphere (128.58 µg Urea N hydrolyzed g<sup>-1</sup> dry soil hour<sup>-1</sup>). Effect of cultivars and interaction between cultivars, growth stages on the urease enzyme activity of the *Bt* and non *Bt* cotton rhizosphere soil sample were not significantly differed (Table 3).

### DISCUSSION

There has been strong debate on the safety of genetically modified plants ever since the introduction onto the market of plant products deriving from transgenic crops. This debate is still very much alive, and several issues have been raised, including the safety of transgenic food and the environmental impact of transgenic plants. The fires of the debate are mainly fed by the insufficient knowledge available on biological systems, and by the potential danger that specific genetic manipulations could give unexpected effects (Saxena *et al.* 2002). The rhizosphere environment hosts a complex microorganism network which interacts very closely with plant roots; the outcome

of such interaction being seen in the strong influence of plant type on the microbial ecosystem of the soil. On considering the different exudates pattern of *Bt* cotton with respect to non transgenic cotton, we were prompted to evaluate whether this unexpected characteristic could affect the functional activity of *T. viride* and *P. Fluorescens*.

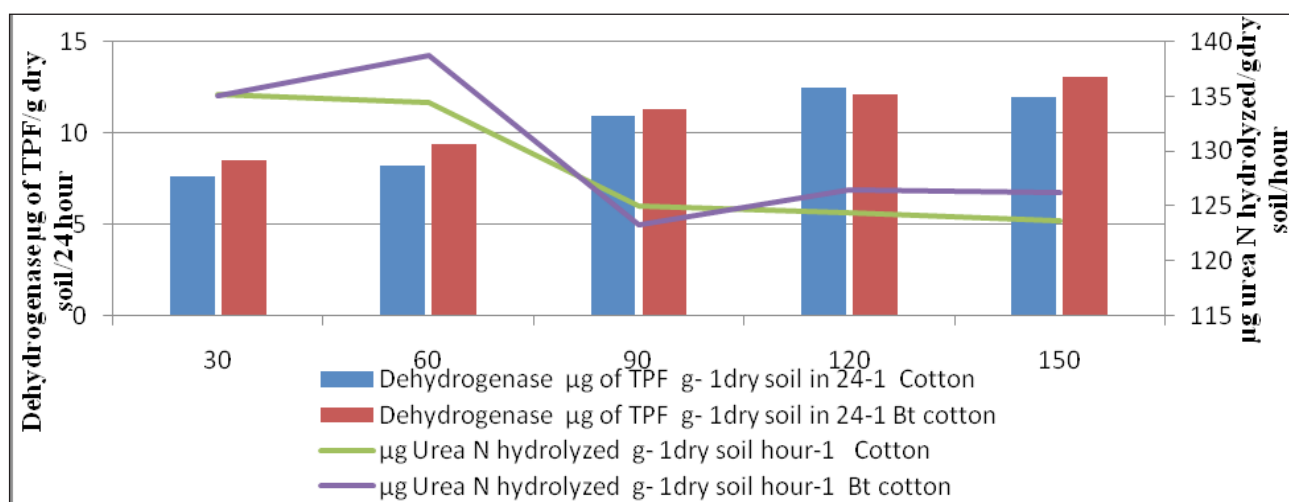


**Fig. 2:** *Rhizoctonia solani* inhibition by *T. viride* and *P. fluorescens* isolates recovered from different intervals from Non *Bt* and *Bt* rhizosphere soils

Inhibition of *R. solani* was observed in present study ranged from 59.2 to 62.8 and 49.4 to 51.6 by *T. viride* and *P. fluorescens* respectively (Fig. 2). Similar results were obtained for suppression of *R. solani* with the use *T. viride* and *P. fluorescens* by several research workers (Chaube and Sharma 2002; Rini and Sulochana 2007; Singh *et al.* 2008; Dev and Dawande 2010). However, no significant difference was observed between *Bt* and non *Bt* cotton. In the present investigation there was no significant difference in the antagonistic activity of *T. viride* and *P. fluorescens* recovered from *Bt* and non *Bt* cotton rhizosphere at different intervals. This might be due to null impact of *Bt* protein on the population level of biocontrol agents. So, the recovered population

**Table 3:** Analysis of dehydrogenase and urease enzyme in rhizosphere soil of *Bt* and non-*Bt* cotton

Sl. No.	Sample taken (DAS)	Dehydrogenase µg of TPF g <sup>-1</sup> dry soil in 24 <sup>-1</sup>			µg Urea N hydrolyzed g <sup>-1</sup> dry soil hour <sup>-1</sup>		
		Cotton	<i>Bt</i> cotton	Mean	Cotton	<i>Bt</i> cotton	Mean
1	30	7.6	8.5	8.1	135.2	135.0	135.1
2	60	8.2	9.4	8.8	134.5	138.7	136.6
3	90	9.9	11.3	11.1	125.1	123.3	124.2
4	120	10.5	12.1	12.3	124.4	126.5	125.5
5	150	11.2	13.1	12.6	123.7	126.2	124.9
Mean		9.48	10.1		128.58	129.94	
		<b>T</b>	<b>C</b>	<b>T × C</b>	<b>T</b>	<b>C</b>	<b>T × C</b>
S.Ed		0.20	0.13	0.29	2.58	1.63	3.65
CD (P=0.05)		0.42	0.27	0.60	5.42	NS	NS



**Fig. 3:** Analysis of dehydrogenase and urease enzyme from field rhizosphere cotton soil from 30DAS interval

and inhibition percent result showed that *Bt* protein did not have any adverse action on the biocontrol efficacy of these antagonists within the period of 45 days.

Activities of soil enzymes indicate the direction and strength of all kinds of biochemical processes in soil and act as key biological indicators of soil. Soil enzymes play an essential role in energy transfer, environmental quality, organic matter decomposition, nutrient cycling and crop productivity (Tabatabai and Bremner 1969; Kumar *et al.* 1992). Measurement of enzymes activity in combination with count of number of key microorganisms provides sensitive information of the changes occurring in soil (Brookes 1995). Similarly, either loss or increasing of particular microbial groups of the mesofauna could cause a loss of specific pathways within nutrient cycling processes, thus affecting important biogeochemical pathways.

In present study dehydrogenase and urease activity was high at initial stage and decreased at later stage of plant growth (Fig. 3). Both the enzyme dehydrogenase and urease, enzymes activity were high in *Bt* cotton rhizosphere as compared to non *Bt* rhizosphere. Activity of dehydrogenase was statistically significant in *Bt* cotton rhizosphere soil when compared with Non *Bt* cotton soil. Even though the enzyme activity of urease enzyme was more in *Bt* cotton but the difference was statistically not significant, on par with activities of non *Bt* plants rhizosphere. The enhancement

in activity of dehydrogenase and urease, in *Bt* cotton rhizosphere may be due to altered composition of its root exudates, which have a profound qualitative and quantitative effect on the rhizospheric microorganisms (Schenck 1976).

Same results were observed by the Mina *et al.* (2011) where urease and dehydrogenase activity was low at the later growth stages. The observed results in this investigation are consistent with an experiment conducted with Cry 1A *Bt* cotton (Sukang-103) and non *Bt* cotton (Sumian-12) for the urease, dehydrogenase, alkaline phosphatase and phenol oxidase activity (Shen *et al.* 2005). Most of the enzymes activity between *Bt* and non-*Bt* cotton rhizosphere were non significant. Similarly, Sun *et al.* (2007) observed stimulated activities of urease, phosphatase, invertase and cellulase enzymes by the addition of *Bt* cotton tissues in soil.

The result of the present study is in conformity with the observation of Wu *et al.* (2004) who observed enhanced dehydrogenase activity in soil incubated with *Bt* transgenic rice straw (during 7-14 days of incubation, later on it declined) compared to soil without straw. However, Liu *et al.* (2008) and Oliveira *et al.* (2008) observed no significant differences in dehydrogenase activity in *Bt* maize and *Bt* rice rhizosphere.

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# Effect of Integrated Weed Management Practices on Flowering, Yield and Economics of African Marigold (*Tagetes erecta* L.) cv. Arka Bangara-2

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## ABSTRACT

An experiment was conducted to find out the effect of different weed control methods on growth and flower yield of African marigold (*Tagetes erecta* L.) cv. Arka Bangara-2 at the Department of Floriculture and Landscape Architecture, College of Horticulture, University of Horticultural Sciences, Bagalkot campus, GKV, Bengaluru, Karnataka during the *rabi* and *summer* seasons 2020-21. The experiment consisted of ten treatment combinations of different weed control methods. The results indicated that post-emergent application of imazethapyr (10 SL) @100 g a.i/ ha fb HW at 45 DAP resulted in the significantly delayed flowering, delayed 50 per cent flowering to complete blooming, significantly bigger (6.97 cm) and heavier flower (6.97 g), maximum weight of flowers plant<sup>-1</sup> (126.73 g), flowers yield ha<sup>-1</sup> (136.85 q) and highest B:C ratio (3.98) and it was found to be on par with pre-emergent application of metolachlor (50 EC) @ 1.0 kg a.i/ ha + HW at 45 DAP in both the seasons. The observations of intercrop treatments varied across the seasons and that may be due to variations in intercrops compatibility and performance. The minimum values for these parameters were recorded in unweeded treatment.

## HIGHLIGHTS

- Imazethapyr (10 SL) @100 g a.i/ ha + HW at 45 DAP and metolachlor (50 EC) @ 1.0 kg a.i/ ha + HW at 45 DAP found best treatments for weed management in marigold.
- Chemical weed management found to be superior to intercropping method.
- Radish found to be suitable intercrop in marigold.
- Intercrop performance varies with season.
- Intercropping is an eco-friendly and sustainable approach for weed management in marigold.

**Keywords:** *Pendimethalin, Oxyfluorfen, Imazethapyr, Metolachlor, Ethoxysulfuron, Fenoxaprop-p-ethyl* and *unweeded*

Marigold (*Tagetes erecta* L.) is one of the most important annual flower crops cultivated commercially in India and Karnataka state for making garlands, religious offering and cut flower

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purposes. Weed competition creates a significant drop in agricultural output. In case of marigold also growth in the initial phases are hampered by weed infestation, which subsequently reduces crop yield. Weeds have been a great nuisance in production of flower yield of marigold which compete heavily with crop plants for light, space, nutrient and moisture and thus caused heavy reduction in their yield. Weed species as well as abundance have a huge impact on the extent of agricultural yield reduction. The removal of weeds either by mechanical means or herbicides during the critical period of crop-weed competition will certainly help in increasing the crop growth and flower yield in marigold. While focusing on best ways to keep weeds at bay we also started to face herbicide hazards. We also need to evolve ecofriendly approaches for sustainable agriculture; one such method for weed control is 'weed smothering intercrops'. Keeping the above facts in view, the present investigation was carried-out to find out the appropriate method of weed management and ascertain a good flower yield in marigold crop.

## MATERIALS AND METHODS

The study was carried out at College of Horticulture, University of Horticultural Sciences, campus GKVK Bengaluru during *Rabi* and *Summer* seasons of 2020-21. There were ten treatments *viz.*, T<sub>1</sub>: Pendimethalin (Extra *i.e.*, 38.7 CS) @ 0.7 kg *a.i* ha<sup>-1</sup> (PRE) + HW at 45 DAP, T<sub>2</sub>: Oxyfluorafen (23.5 EC) @ 0.1 kg *a.i* ha<sup>-1</sup> (PRE) + HW at 45 DAP, T<sub>3</sub>: Metolachlor (50 EC) @ 1.0 kg *a.i* ha<sup>-1</sup> (PRE) + HW at 45 DAP, T<sub>4</sub>: Imazethapyr (10 SL) @100 g *a.i* ha<sup>-1</sup> (POE) + HW at 45 DAP, T<sub>5</sub>: Ethoxysulfuran (15 WG) @ 12 g *a.i* ha<sup>-1</sup> (POE) + HW at 45 DAP, T<sub>6</sub>: Fenoxaprop-p-ethyl (9 EC) @ 67.5 g *a.i* ha<sup>-1</sup> (POE) + HW at 45 DAP, T<sub>7</sub>: Marigold + radish (intercrop) + HW at 45 DAP, T<sub>8</sub>: Marigold + carrot (intercrop) + HW at 45 DAP, T<sub>9</sub>: Unweeded check and T<sub>10</sub>: Weed free check. The experiment was laid out in Randomized Complete Block Design (RBD) with three replications having plot size of 4.2 m × 3.15 m. All recommended cultural practices were followed as on when required. The transplanting of the seedlings was done at the spacing of 60 × 45 cm. Pre-emergence herbicides application and intercrop sowing was done at three days after transplanting and post-emergence herbicides were applied at 15 days after transplanting. The observations were

recorded at various intervals following standard methodology.

## RESULTS AND DISCUSSION

Competition of weeds with the crop for light, space and nutrients reduces the growth components to a greater extent. Decreased growth components will have major impact on flowering parameters affecting yield. In this investigation also, flowering parameters affected drastically at an indirect proportion to weed gradient in different plots due to competition by weeds. In general, weed control treatments resulted in significantly superior but delayed flowering behavior as compared to unweeded check (Table 1).

Among different weed control treatments during *Rabi* season post-emergent application of imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP produced significantly delayed flowering (70.33 days), delayed 50 per cent flowering (101.99 days), delayed full bloom (136.63 days) and longer duration of flowering (79.97 days). However, it was found on par with pre-emergence application of metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP for flower initiation (69.00 days), days to 50 per cent flowering (98.66 days), days to full bloom (135.66 days) and flowering duration (73.66 days). This might be due to the hypothesis that, early flowering is an indication of poor growth and lack of nutrients availability making the plant to bloom early because of stress.

Similarly, during *Summer* season post-emergent application of imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP flowered significantly later (68.12 days), days to 50 per cent flowering (98.19 days), days to full bloom (137.64 days) and flowering duration (76.35 days). However, it was found on par with and pre-emergence application of metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP for flowering initiation (64.30 days), 50 per cent flowering (95.56 days), full bloom (133.10 days) and flowering duration (74.19 days). Comparatively delayed flowering is seen in *Summer* season crop indicating poor performance of marigold in high temperature conditions.

Weed control treatments influenced significant impact on flowering parameters, that may be due



**Table 1:** Effect of different weed control treatments on flowering parameters of marigold at different intervals during *Rabi* and *Summer* season

Treatments	Days to flower initiation		Days to 50% flowering		Days to full bloom		Duration of flowering	
	<i>Rabi</i>	<i>Summer</i>	<i>Rabi</i>	<i>Summer</i>	<i>Rabi</i>	<i>Summer</i>	<i>Rabi</i>	<i>Summer</i>
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + HW at 45 DAP	62.33	59.21	93.50	91.26	125.20	122.83	69.60	67.79
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + HW at 45 DAP	60.33	55.67	90.69	86.83	119.65	113.10	65.66	58.19
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + HW at 45 DAP	69.00	64.30	98.66	95.56	135.66	133.10	73.66	74.79
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + HW at 45 DAP	70.33	68.12	101.99	98.19	136.63	137.64	79.97	76.35
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + HW at 45 DAP	52.33	51.00	75.46	78.39	91.12	89.29	47.56	43.84
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + HW at 45 DAP	61.67	58.67	91.32	90.16	122.33	119.51	67.87	62.93
T <sub>7</sub> : Marigold + radish (intercrop) + HW at 45 DAP	59.00	54.33	84.59	82.20	109.23	108.20	60.23	57.72
T <sub>8</sub> : Marigold + carrot (intercrop) + HW at 45 DAP	57.67	53.33	83.32	81.09	93.20	92.14	48.49	44.38
T <sub>9</sub> : Unweeded check	51.67	50.33	71.72	70.83	90.43	87.06	45.53	42.66
T <sub>10</sub> : Weed free check	76.67	71.67	105.69	101.10	138.33	139.61	82.16	80.95
S. Em ±	3.71	3.51	3.34	2.66	3.28	3.78	3.38	3.16
CD @ 5%	11.12	10.54	10.02	7.97	9.83	11.34	10.15	9.48
CV	12.17	10.54	13.72	11.68	12.59	12.42	11.27	13.85

to better performance of herbicides to control weeds effectively in both seasons compared to unweeded check and in turn weeds competition with crop for resources is reduced and crop acquired more amount of nutrients and growth is enhanced promoting more vegetative growth and delaying flowering (Markam *et al.* (2020), Gamit *et al.* (2019) and Kumar *et al.* (2010) in marigold crop).

Competition of weeds with the crop for resources reduces the growth and flowering attributes to a greater extent. In this study also, reduced growth and flowering parameters affected yield drastically in unweeded plot. In general weed control treatments resulted in significantly higher yield attributes as compared to unweeded check (Table 2).

Post-emergent application of imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP showed significantly higher number of flowers per plant (104.33), flower yield per plant (0.68 kg plant<sup>-1</sup>), flower yield per plot (29.84 kg plot<sup>-1</sup>) and flower yield per hectare (22.55 t ha<sup>-1</sup>) among different weed control treatments. However, it was found on par with pre-emergence application of

metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP for number of flowers per plant (101.00), flower yield per plant (0.63 kg plant<sup>-1</sup>), flower yield per plot (27.73 kg plot<sup>-1</sup>) and flower yield per hectare (20.96 t ha<sup>-1</sup>) during *Rabi* season.

Similarly, during *Summer* season significantly higher number of flowers per plant (98.26), flower yield per plant (0.59 kg plant<sup>-1</sup>), flower yield per plot (25.98 kg plot<sup>-1</sup>) and flower yield per hectare (19.64 t ha<sup>-1</sup>) were registered with post-emergent application of imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP. Which was found on par with pre-emergence application of metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP for number of flowers per plant (94.00), flower yield per plant (0.53 kg plant<sup>-1</sup>), flower yield per plot (23.20 kg plot<sup>-1</sup>) and flower yield per hectare (17.54 t ha<sup>-1</sup>).

With the best weed control treatments significantly higher number of flowers per plant was recorded as the crop and weed competition for resources is less and crop acquired more amounts of nutrients and growth is enhanced, that made the crop to

**Table 2:** Effect of different weed control treatments on yield parameters of marigold at different intervals during *Rabi* and *Summer* season

Treatments	Number of flowers per plant		Flower yield per plant (kg plant <sup>-1</sup> )		Flower yield per plot (kg plot <sup>-1</sup> )		Flower yield per hectare (t ha <sup>-1</sup> )	
	<i>Rabi</i>	<i>Summer</i>	<i>Rabi</i>	<i>Summer</i>	<i>Rabi</i>	<i>Summer</i>	<i>Rabi</i>	<i>Summer</i>
	T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + HW at 45 DAP	97.00	90.67	0.56	0.47	24.75	20.83	18.71
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + HW at 45 DAP	88.00	82.00	0.38	0.30	16.73	13.24	12.64	10.01
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + HW at 45 DAP	101.00	94.00	0.63	0.53	27.73	23.20	20.96	17.54
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + HW at 45 DAP	104.33	98.26	0.68	0.59	29.84	25.98	22.55	19.64
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + HW at 45 DAP	35.00	31.00	0.11	0.10	4.74	4.56	3.59	3.44
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + HW at 45 DAP	95.50	93.20	0.51	0.45	22.27	19.77	16.83	14.94
T <sub>7</sub> : Marigold + radish (intercrop) + HW at 45 DAP	60.50	55.20	0.21	0.20	9.32	8.77	7.04	6.63
T <sub>8</sub> : Marigold + carrot (intercrop) + HW at 45 DAP	43.50	40.10	0.13	0.14	5.93	6.18	4.48	4.67
T <sub>9</sub> : Unweeded check	23.00	21.20	0.05	0.06	2.33	2.43	1.76	1.83
T <sub>10</sub> : Weed free check	112.67	101.00	0.74	0.64	32.77	27.95	24.77	21.13
S. Em ±	4.55	3.05	0.05	0.04	2.03	1.81	1.71	1.59
CD @ 5%	13.65	9.14	0.15	0.13	6.10	5.44	5.12	4.76
CV	11.67	11.62	12.23	12.28	11.23	12.28	13.23	12.25

branch more and with wider leaf area number of flowers increased in both the seasons compared to unweeded check (Markam *et al.* (2020) in marigold, Gamit *et al.* (2019) in marigold and Jain *et al.* (2015) in tuberose). With respect to radish intercrop growth and flowering recovery can be correlated with similar studies from Thomas *et al.* (2018), in which the contrasting results were obtained for carrot intercrop where, it drastically suppressed main crop during *Rabi* season and failed to control weeds in *Summer* leading to yield loss of both marigold and carrot.

Gross returns, net returns and B: C of marigold in both the seasons found to vary with respect to different weed control treatments and remained unaltered across the seasons.

In the present study, all weed management practices achieved a higher net returns and B:C ratio over weedy check. Economic analysis of data revealed that, among different weed management practices, higher net returns was noticed in the weed free check (4,51,751 ₹ ha<sup>-1</sup> with B:C ratio of 2.55) which was followed by post-emergent application of

imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP (3,97,171 ₹ ha<sup>-1</sup> with B:C ratio of 2.42) and pre-emergence application of metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP (3,49,551 ₹ ha<sup>-1</sup> with B:C ratio of 2.25).

Similarly in *Summer* also, all weed management practices achieved a higher net returns and B:C ratio over weedy check. Economic analysis of data revealed that, among different weed management practices, higher net returns was noticed in the weed free check (3,42,551 ₹ ha<sup>-1</sup> with B:C ratio of 2.18) which was followed by post-emergent application of imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP (3,09,871 Rs ha<sup>-1</sup> with B:C ratio of 2.11) and pre-emergence application of metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP (2,46,951 Rs ha<sup>-1</sup> with B:C ratio of 1.88). These results are in line with findings of Shalini and Patil (2006) in tuberose. Although higher flower yield was realized in weed free check, due to higher cost of cultivation in terms of higher requirement of labours for



weeding, in the present scenario there is scarcity of agricultural labour. Pre-emergence application of metolachlor 50% EC @ 1.0 kg a.i ha<sup>-1</sup> followed by hand weeding at 45 DAP and post-emergent application of imazethapyr 10% SL @ 100 g a.i ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP can be suggested for better weed control and good returns in marigold. Marigold intercropped with radish fb hand weeding at 45 DAP can be suggested for sustainable eco-friendly agriculture with optimum returns (2,01,880 and 1,13,751 ₹ ha<sup>-1</sup>) and maintaining biodiversity with functional biodiversity conservation approach in both *Rabi* and *Summer* seasons.

## CONCLUSION

Different weed control treatments significantly influenced the growth of marigold. Significantly, taller plant height, broader plant spread (E-W and N-S), number of primary and secondary branches, leaf area, plant dry weight at grand growth stages, significantly delayed flowering with all higher yield parameters proved with highest B:C ratio were recorded with weed free treatment which was found to be on par with post-emergent application of imazethapyr (10 SL) @100 g a.i/ ha + HW at 45 DAP and pre-emergent application of metolachlor (50 EC) @ 1.0 kg a.i/ ha + HW at 45 DAP. Hence, application of imazethapyr (10 SL) @100 g a.i/ ha + HW at 45 DAP and metolachlor (50 EC) @ 1.0 kg a.i/ ha + HW at 45 DAP can be recommended as better weed management approaches to farmers.

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# Impact of Integrated Weed Management Strategies on Growth and Yield of African Marigold (*Tagetes erecta* L.) cv. Arka Bangara-2

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## ABSTRACT

An experiment was conducted to find out the effect of different weed control methods on growth, and flower yield of African marigold (*Tagetes erecta* L.) cv. Arka Bangara-2 at the Department of Floriculture and Landscape Architecture, College of Horticulture, University of Horticultural Sciences, Bagalkot campus, GKV, Bengaluru, Karnataka during the *rabi* and *summer* seasons 2020-21. The experiment consisted of ten treatment combinations of different weed control methods. The results indicated that weed free treatment resulted in the maximum plant height, number of primary and secondary branches plant<sup>-1</sup>, maximum plant spread in E-W and N-S, significantly bigger flower (6.21 and 6.12 cm, respectively), maximum weight of flowers plant<sup>-1</sup> (0.74 and 0.64 kg, respectively), flowers yield ha<sup>-1</sup> (24.77 and 21.13 t, respectively) and it was found to be on par with post-emergent application of imazethapyr (10 SL) @100 g a.i/ ha fb HW at 45 DAP and pre-emergent application of metolachlor (50 EC) @ 1.0 kg a.i/ ha + HW at 45 DAP in both the seasons. The observations of intercrop treatments varied across the seasons and that may be due to variations in intercrops adoptability and performance. The minimum values for these parameters were recorded in unweeded treatment.

## HIGHLIGHTS

- Imazethapyr (10 SL) @100 g a.i/ ha + HW at 45 DAP and metolachlor (50 EC) @ 1.0 kg a.i/ha + HW at 45 DAP found best treatments for weed management in marigold.
- Chemical weed management found to be superior to intercropping method.
- Intercrop performance varies with seasons which also hinder or influence main crop performance.
- Intercropping is an eco-friendly and sustainable approach for weed management in marigold.

**Keywords:** Pendimethalin, Oxyfluorfen, Imazethapyr, Metolachlor, Ethoxysulfuron, Fenoxaprop-p-ethyl and unweeded

One of the most significant annual flower crops is African marigold (*Tagetes erecta* L.), which is grown commercially in India and the state of Karnataka for garlands, religious offerings and cut flower

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uses. Weed competition creates a significant drop in agricultural output. In case of marigold also growth in the initial phases are hampered by weed infestation, which subsequently reduces crop yield. Weeds have been a great nuisance in production of flower yield of marigold which compete heavily with crop plants for light, space, nutrient and moisture and thus caused heavy reduction in their yield. Weed species as well as abundance have a huge impact on the extent of agricultural yield reduction. The removal of weeds either by mechanical means or herbicides during the critical period of crop-weed competition will certainly help in increasing the crop growth and flower yield in marigold. While focusing on best ways to keep weeds at bay we also started to face herbicide hazards. We also need to evolve ecofriendly approaches for sustainable agriculture; one such method for weed control is 'weed smothering intercrops'. Keeping the above facts in view, the present investigation was carried-out to find out the appropriate method of weed management and ascertain a good flower yield in marigold crop.

## MATERIALS AND METHODS

The study was carried out at College of Horticulture, University of Horticultural Sciences, campus GKVK Bengaluru during *Rabi* and *Summer* seasons of 2020-21. There were ten treatments *viz.*, T<sub>1</sub>: Pendimethalin (Extra *i.e.*, 38.7 CS) @ 0.7 kg *a.i* ha<sup>-1</sup> (PRE) + HW at 45 DAP, T<sub>2</sub>: Oxyfluorafen (23.5 EC) @ 0.1 kg *a.i* ha<sup>-1</sup> (PRE) + HW at 45 DAP, T<sub>3</sub>: Metolachlor (50 EC) @ 1.0 kg *a.i* ha<sup>-1</sup> (PRE) + HW at 45 DAP, T<sub>4</sub>: Imazethapyr (10 SL) @100 g *a.i* ha<sup>-1</sup> (POE) + HW at 45 DAP, T<sub>5</sub>: Ethoxysulfuran (15 WG) @ 12 g *a.i* ha<sup>-1</sup> (POE) + HW at 45 DAP, T<sub>6</sub>: Fenoxaprop-p-ethyl (9 EC) @ 67.5 g *a.i* ha<sup>-1</sup> (POE) + HW at 45 DAP, T<sub>7</sub>: Marigold + radish (intercrop) + HW at 45 DAP, T<sub>8</sub>: Marigold + carrot (intercrop) + HW at 45 DAP, T<sub>9</sub>: Unweeded check and T<sub>10</sub>: Weed free check. The experiment was laid out in Randomized Complete Block Design (RBD) with three replications having plot size of 4.2 m × 3.15 m. All recommended cultural practices were followed as on when required. The transplanting of the seedlings was done at the spacing of 60×45 cm. Pre-emergence herbicides application and intercrop sowing was done at three days after transplanting and post-emergence herbicides were applied at 15 days after transplanting. The observations were

recorded at various intervals following standard methodology.

## RESULT AND DISCUSSION

The predominant weed flora observed in the experimental field in association with the marigold crop were grasses *viz.*, *Digitaria ischaemum* (Schreb.) Muhl., *Digitaria sanguinalis* L. Scop., *Commelina benghalensis* L. and *Echinochloa crusgalli* L.; among broad leaved weeds *Portulaca oleracea* L., *Stachytarpheta indica* L. Vahl., *Alternanthera sessilis* L., *Alternanthera philoxeroides* (Mart.) Griseb., *Taraxacum officinale* F. H. Wigg., *Parthenium hysterophorous* L., *Ageratum conyzoides* L., *Euphorbia heterophylla* L., *Ipomea triloba* L. and *Gallinsoga parviflora* Cav. These results are in line with reports of Shwetha *et al.* 2016, Pujeri and Hosmath, 2019; Markam *et al.* 2020 and Nagapushpa *et al.* 2021.

Weeds compete with the crop for resources and reduce the growth as well as growth components to a greater extent. In this investigation also, growth parameters reduced drastically in unweeded plot due to competition by weeds. In general different weed control treatments resulted in significantly higher growth components compared to unweeded check at 30, 45, 60, 90 DAP and grand growth stage (Table 1-10).

Among different weed control treatments post-emergent application of imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by HW at 45 DAP recorded significantly higher growth parameters at 30, 45, 60, 90 DAP and grand growth stage *viz.*, plant height (18.03, 28.20, 32.27, 43.77 and 49.40 cm, respectively), plant spread E-W (17.12, 26.20, 29.20, 40.97 and 48.80 cm, respectively), plant spread N-S (17.56, 25.53, 29.07, 40.47 and 47.80 cm, respectively), number of primary branches (2.44, 3.87, 4.18, 4.52 and 4.60, respectively), number of secondary branches (8.50, 14.90, 29.70, 47.06 and 54.29, respectively), leaf area (11.96 cm<sup>2</sup>) and dry weight (46.20 g plant<sup>-1</sup>) at grand growth stages. However, it was found on par with pre-emergence application of metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP for plant height (17.31, 27.73, 31.50, 41.43 and 47.93 cm, respectively), plant spread E-W (16.94, 24.93, 27.38, 39.13 and 45.33 cm, respectively), plant spread N-S (16.44, 24.07, 26.33, 38.93 and 45.27 cm, respectively), number of primary branches (2.32, 3.75, 4.06, 4.48 and 4.51,

**Table 1:** Effect of different weed control treatments on plant height (cm) of marigold at different intervals during *Rabi* season

Treatments	Plant height (cm)				Grand growth stage
	30 DAP	45 DAP	60 DAP	90 DAP	
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	15.41	25.20	29.89	38.37	44.30
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	14.41	24.07	28.57	36.57	42.80
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	17.31	27.73	31.50	41.43	47.93
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	18.03	28.20	32.27	43.77	49.40
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	12.47	20.73	25.13	34.77	40.07
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	15.11	24.53	29.72	37.43	42.93
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	13.39	21.87	28.33	35.83	41.53
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	13.03	21.60	25.93	35.73	41.00
T <sub>9</sub> : Unweeded check	12.00	20.60	21.73	33.40	38.67
T <sub>10</sub> : Weed free check	18.18	28.47	32.93	46.13	55.53
S. Em ±	0.33	0.28	0.44	1.64	2.84
CD @ 5%	0.98	0.85	1.32	4.92	8.52
CV	12.20	11.95	11.21	12.03	12.51

**Table 2:** Effect of different weed control treatments on plant height (cm) of marigold at different intervals during *Summer* season

Treatments	Plant height (cm)				Grand growth stage
	30 DAP	45 DAP	60 DAP	90 DAP	
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	15.10	21.07	25.84	30.52	34.23
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	14.20	19.51	23.13	28.91	32.28
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	16.78	24.31	28.34	33.49	38.07
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	17.00	25.55	29.56	35.39	40.85
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	12.78	17.84	18.07	23.26	24.91
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	14.43	20.39	24.20	29.12	33.71
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	13.41	18.91	22.40	28.55	31.53
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	13.13	18.41	21.20	28.29	30.64
T <sub>9</sub> : Unweeded check	11.88	15.49	17.61	21.94	23.53
T <sub>10</sub> : Weed free check	18.41	27.53	31.26	37.00	41.18
S. Em ±	0.63	1.64	1.08	1.77	1.90
CD @ 5%	1.88	4.92	3.25	5.31	5.70
CV	12.97	11.66	11.26	11.99	12.39

**Table 3:** Effect of different weed control treatments on plant spread E-W (cm) of marigold at different intervals during *Rabi* season

Treatments	Plant spread E-W (cm)				
	30 DAP	45 DAP	60 DAP	90 DAP	Grand growth stage
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	13.84	21.40	23.60	34.73	42.07
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	13.12	19.80	22.93	32.53	40.47
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	16.94	24.93	27.38	39.13	45.33
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	17.12	26.20	29.20	40.97	48.80
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	9.11	16.40	21.60	30.03	32.60
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	13.56	20.22	23.12	34.27	41.12
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	12.45	19.67	22.27	31.17	40.40
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	11.21	18.80	21.60	30.67	38.40
T <sub>9</sub> : Unweeded check	8.47	13.80	16.60	27.03	29.73
T <sub>10</sub> : Weed free check	18.50	26.60	30.27	44.33	56.40
S. Em ±	1.21	0.97	1.17	2.37	4.05
CD @ 5%	3.62	2.91	3.50	7.12	12.14
CV	12.67	11.37	11.03	11.24	10.63

**Table 4:** Effect of different weed control treatments on plant spread E-W (cm) of marigold at different intervals during *Summer* season

Treatments	Plant spread E-W (cm)				
	30 DAP	45 DAP	60 DAP	90 DAP	Grand growth stage
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	13.23	18.91	22.13	30.04	35.24
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	12.22	17.50	21.21	28.80	32.63
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	14.26	21.20	25.54	38.71	41.75
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	14.83	22.50	26.51	40.70	46.47
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	9.27	14.29	18.38	23.59	27.57
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	12.48	17.65	21.79	29.43	34.13
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	11.94	15.87	19.50	27.02	31.00
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	10.17	15.47	18.74	24.62	29.05
T <sub>9</sub> : Unweeded check	8.28	14.05	17.77	22.81	26.83
T <sub>10</sub> : Weed free check	14.95	24.13	29.45	42.93	51.63
S. Em ±	0.56	1.29	2.04	2.72	3.77
CD @ 5%	1.68	3.87	6.11	8.17	11.32
CV	12.21	12.26	13.37	11.98	12.40



**Table 5:** Effect of different weed control treatments on plant spread N-S (cm) of marigold at different intervals during *Rabi* season

Treatments	Plant spread N-S (cm)				
	30 DAP	45 DAP	60 DAP	90 DAP	Grand growth stage
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	13.81	22.10	24.00	34.50	41.20
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	12.44	21.40	22.73	33.08	39.27
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	16.44	24.07	26.33	38.93	45.27
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	17.56	25.53	29.07	40.47	47.80
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	10.59	17.27	20.73	28.87	37.20
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	13.38	21.67	23.13	33.53	39.40
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	11.96	19.53	21.75	32.57	37.93
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	11.52	19.20	21.53	30.63	37.87
T <sub>9</sub> : Unweeded check	8.23	13.53	16.00	26.43	30.47
T <sub>10</sub> : Weed free check	19.39	27.20	30.13	43.07	56.20
S. Em ±	1.25	1.27	1.91	1.95	3.80
CD @ 5%	3.75	3.81	5.72	5.86	11.41
CV	12.43	11.49	10.85	12.54	10.74

**Table 6:** Effect of different weed control treatments on plant spread N-S (cm) of marigold at different intervals during *Summer* season

Treatments	Plant spread N-S (cm)				
	30 DAP	45 DAP	60 DAP	90 DAP	Grand growth stage
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	12.50	20.19	27.70	29.33	33.77
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	11.64	18.50	25.93	27.77	30.88
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	14.99	23.43	30.11	33.97	39.96
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	15.97	25.08	31.00	35.13	41.55
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	9.53	14.40	21.07	24.63	28.50
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	12.26	19.23	26.70	28.97	32.77
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	11.32	18.27	22.47	27.50	30.25
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	10.24	15.93	22.07	25.30	29.63
T <sub>9</sub> : Unweeded check	9.47	13.93	19.82	24.48	26.71
T <sub>10</sub> : Weed free check	16.57	25.57	33.04	37.87	43.46
S. Em ±	0.97	1.15	1.71	1.63	2.18
CD @ 5%	2.91	3.45	5.12	4.89	6.53
CV	11.72	12.74	11.16	12.84	12.77

**Table 7:** Effect of different weed control treatments on number of primary branches of marigold at different intervals during *Rabi* season

Treatments	No. of primary branches				
	30 DAP	45 DAP	60 DAP	90 DAP	Grand growth stage
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	2.12	3.50	3.75	4.07	4.10
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	2.03	3.33	3.60	3.63	3.67
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	2.32	3.75	4.06	4.48	4.51
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	2.44	3.87	4.18	4.52	4.60
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	1.87	2.93	3.13	3.23	3.47
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	2.05	3.41	3.63	3.84	4.07
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	1.96	3.27	3.50	3.47	3.60
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	1.95	2.93	3.40	3.40	3.50
T <sub>9</sub> : Unweeded check	1.81	2.73	3.10	3.10	3.20
T <sub>10</sub> : Weed free check	2.62	4.13	4.20	4.57	5.20
S. Em ±	0.10	0.16	0.15	0.15	0.26
CD @ 5%	0.31	0.49	0.44	0.46	0.78
CV	12.85	12.46	11.53	12.60	13.23

**Table 8:** Effect of different weed control treatments on number of primary branches of marigold at different intervals during *Summer* season

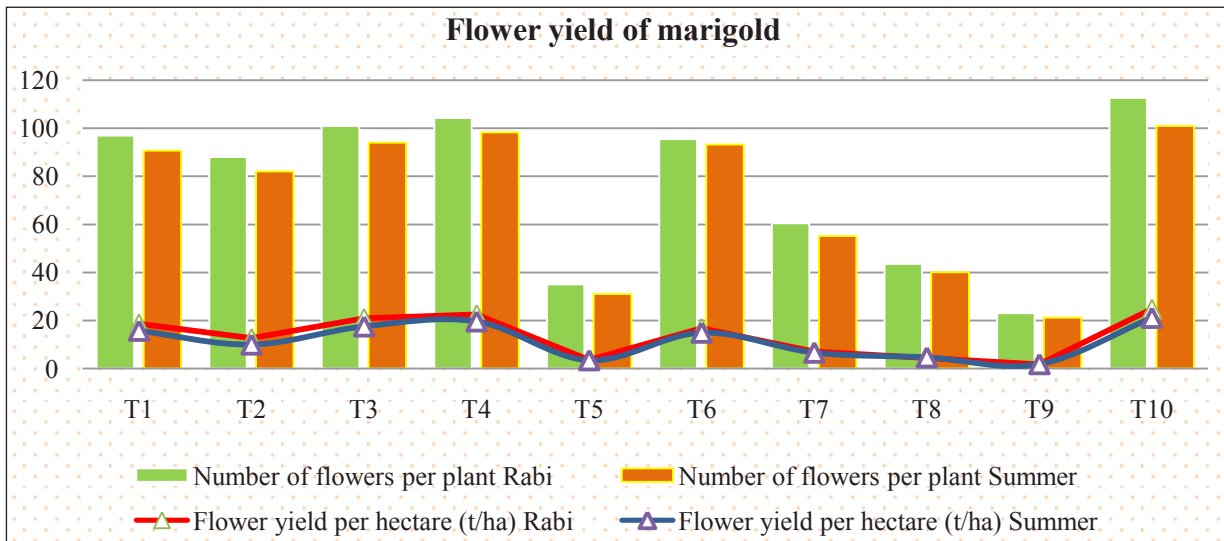
Treatments	No. of primary branches				
	30 DAP	45 DAP	60 DAP	90 DAP	Grand growth stage
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	2.23	3.37	3.79	4.02	4.27
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	2.13	3.33	3.65	3.85	3.90
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	2.61	3.88	4.19	4.45	4.83
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	2.75	3.95	4.38	4.67	5.00
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	1.82	2.90	3.37	3.50	3.63
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	2.18	3.36	3.72	3.90	4.20
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	1.94	3.23	3.63	3.80	3.83
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	1.92	3.13	3.43	3.53	3.80
T <sub>9</sub> : Unweeded check	1.71	2.83	3.37	3.50	3.60
T <sub>10</sub> : Weed free check	2.80	4.03	4.47	4.80	5.43
S. Em ±	0.10	0.17	0.20	0.21	0.29
CD @ 5%	0.30	0.52	0.60	0.64	0.87
CV	11.94	12.45	11.82	10.11	12.71

**Table 9:** Effect of different weed control treatments on number of secondary branches of marigold at different intervals during *Rabi* season

Treatments	No. of secondary branches				Grand growth stage
	30 DAP	45 DAP	60 DAP	90 DAP	
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	7.15	12.17	25.52	37.10	41.82
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	7.01	11.00	24.07	34.80	38.74
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	8.18	13.63	27.80	43.63	49.13
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	8.50	14.90	29.70	47.06	54.29
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	4.32	7.68	19.67	26.03	34.85
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	7.08	11.73	25.31	36.93	40.84
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	6.46	10.50	23.61	30.04	37.69
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	6.19	9.40	20.60	28.97	35.16
T <sub>9</sub> : Unweeded check	4.25	6.63	10.37	17.33	22.31
T <sub>10</sub> : Weed free check	8.87	15.57	31.87	53.77	59.11
S. Em ±	0.32	0.85	1.44	3.61	4.77
CD @ 5%	0.95	2.54	4.31	10.83	14.32
CV	10.05	11.25	12.46	12.95	11.63

**Table 10:** Effect of different weed control treatments on number of secondary branches of marigold at different intervals during *Summer* season

Treatments	No. of secondary branches				Grand growth stage
	30 DAP	45 DAP	60 DAP	90 DAP	
T <sub>1</sub> : Pendimethalin (Extra <i>i.e.</i> , 38.7 CS) @ 0.7 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	6.95	10.27	17.53	24.50	31.22
T <sub>2</sub> : Oxyfluorafen (23.5 EC) @ 0.1 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	6.01	10.03	16.20	22.00	29.52
T <sub>3</sub> : Metolachlor (50 EC) @ 1.0 kg <i>a.i</i> ha <sup>-1</sup> (PRE) + one HW at 45 DAP	7.58	11.83	20.30	34.00	40.81
T <sub>4</sub> : Imazethapyr (10 SL) @100 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	7.70	13.20	22.23	35.93	45.29
T <sub>5</sub> : Ethoxysulfuran (15 WG) @ 12 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	5.41	8.73	12.67	17.33	22.84
T <sub>6</sub> : Fenoxaprop-p-ethyl (9 EC) @ 67.5 g <i>a.i</i> ha <sup>-1</sup> (POE) + one HW at 45 DAP	6.53	10.12	17.40	23.43	30.82
T <sub>7</sub> : Marigold + radish (intercrop) + one HW at 45 DAP	5.78	9.67	14.00	18.77	28.84
T <sub>8</sub> : Marigold + carrot (intercrop) + one HW at 45 DAP	5.70	8.73	13.20	17.90	24.49
T <sub>9</sub> : Unweeded check	4.10	6.67	8.20	13.73	14.31
T <sub>10</sub> : Weed free check	8.75	13.60	22.93	40.43	51.11
S. Em ±	0.47	0.91	1.37	4.17	5.07
CD @ 5%	1.42	2.74	4.12	12.41	15.20
CV	10.10	12.81	12.36	11.45	11.92



T<sub>1</sub>: Pendimethalin (Extra i.e. 38.7 CS) @ 0.7 kg a.i ha<sup>-1</sup> (PRE) + HW at 45 DAP; T<sub>2</sub>: Oxyfluorafen (23.5 EC) @ 0.1 kg a.i ha<sup>-1</sup> (PRE) + HW at 45 DAP; T<sub>3</sub>: Metolachlor (50 EC) @ 1.0 kg a.i ha<sup>-1</sup> (PRE) + HW at 45 DAP; T<sub>4</sub>: Imazethapyr (10 SL) @ 100 g a.i ha<sup>-1</sup> (POE) + HW at 45 DAP; T<sub>5</sub>: Ethoxysulfuran (15 WG) @ 12 g a.i ha<sup>-1</sup> (POE) + HW at 45 DAP; T<sub>6</sub>: Fenoxaprop-p-ethyl (9 EC) @ 67.5 g a.i ha<sup>-1</sup> (POE) + HW at 45 DAP; T<sub>7</sub>: Marigold + Radish (Intercrop) + HW at 45 DAP; T<sub>8</sub>: Marigold + Carrot (Intercrop) + HW at 45 DAP; T<sub>9</sub>: Unweeded check; T<sub>10</sub>: Weed free check.

**Fig. 1:** Effect of weed control treatments on number of flowers per plant and yield per ha of marigold in Rabi and Summer season

respectively), number of secondary branches (8.18, 13.63, 27.80, 43.63 and 49.13, respectively), leaf area (10.94 cm<sup>2</sup>) and dry weight (43.58 g plant<sup>-1</sup>) at grand growth stages during *Rabi* season.

Similarly, in *Summer* among different weed control treatments at 30, 45, 60, 90 DAP and grand growth stage post-emergent application of imazethapyr 10% SL @ 100 g a.i ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP treatment registered significantly higher growth parameters *viz.*, plant height (17.00, 25.55, 29.56, 35.39 and 40.85 cm, respectively), plant spread E-W (14.83, 22.50, 26.51, 40.70 and 46.47 cm, respectively), plant spread N-S (15.97, 25.08, 31.00, 35.13 and 41.55 cm, respectively), number of primary branches (2.75, 3.95, 4.38, 4.67 and 5.00, respectively), number of secondary branches (7.70, 13.20, 22.23, 35.93 and 45.29, respectively), leaf area (11.26 cm<sup>2</sup>) and dry weight (42.67 g plant<sup>-1</sup>) at grand growth stage. However, it was found on par with pre-emergence application of metolachlor 50% EC @ 1.0 kg a.i ha<sup>-1</sup> followed by hand weeding at 45 DAP for plant height (16.78, 24.31, 28.34, 33.49 and 38.07 cm, respectively), plant spread E-W (14.26, 21.20, 25.54, 38.71 and 41.75 cm, respectively), plant spread N-S (14.99, 23.43, 30.11, 33.97 and 39.96 cm, respectively), number of primary branches (2.61, 3.88, 4.19, 4.45 and 4.83, respectively), number of

secondary branches (7.58, 11.83, 20.30, 34.00 and 40.81, respectively), leaf area (10.85 cm<sup>2</sup>) and dry weight (39.03 g plant<sup>-1</sup>) at grand growth stage.

In both seasons the results may be due to better performance of herbicides to control weeds effectively reducing weeds competition with crop for resources and crop growth is promoted. These results are in line with findings of Markam *et al.* (2020) in marigold, Vinay and Gowda (2011) in China aster and Kumar *et al.* (2010) in marigold.

Radish intercrop smothers weeds and when its harvested and weeding is done, the marigold main crop resumes its growth and performs better (Nelson *et al.* 1991 and Pawlowski and Wesolowski 1980) at 60, 90 DAP and at grand growth stage. These results are contrasting in carrot intercrop during *Rabi* season where, the intercrop took long duration for crop growth and became dominant over marigold. That may be due to cultivar effect (Minotti 1991 and Callaway 1990) as the carrot is wide diverse crop with 21 different types. The hybrid which we have used is an Asiatic type carrot which grows taller and broader, whereas the European type carrots are short in height and spreads less. Even though carrot controlled weeds effectively; it harvested more nutrients than marigold and suppressed its performance and plant height registered as



low as unweeded check. Carrot failed to perform better in *Summer* as it is cool season crop and hence, there was no weed smothering and hence, weed competition increased resulting in failure of main crop as well as intercrop. However at all the stages, marigold from intercrop treatment showed poor performance compared to better performing herbicide treated plot in general. These results are in line with findings of Suman, (2014). The least of growth parameters at all stages were recorded in unweeded check as the weeds competed with crop for space, light and nutrients.

Competition of weeds with the crop for resources reduces the growth and flowering attributes to a greater extent. In this study also, reduced growth and flowering parameters affected yield drastically in unweeded plot. In general weed control treatments resulted in significantly higher yield attributes as compared to unweeded check.

Post-emergent application of imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP showed significantly higher number of flowers per plant (104.33), flower yield per plant (0.68 kg plant<sup>-1</sup>), flower yield per plot (29.84 kg plot<sup>-1</sup>) and flower yield per hectare (22.55 t ha<sup>-1</sup>) among different weed control treatments. However, it was found on par with pre-emergence application of metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP for number of flowers per plant (101.00), flower yield per plant (0.63 kg plant<sup>-1</sup>), flower yield per plot (27.73 kg plot<sup>-1</sup>) and flower yield per hectare (20.96 t ha<sup>-1</sup>) during *Rabi* season.

Similarly, during *Summer* season significantly higher number of flowers per plant (98.26), flower yield per plant (0.59 kg plant<sup>-1</sup>), flower yield per plot (25.98 kg plot<sup>-1</sup>) and flower yield per hectare (19.64 t ha<sup>-1</sup>) were registered with post-emergent application of imazethapyr 10% SL @ 100 g *a.i* ha<sup>-1</sup> at 15 DAP followed by hand weeding at 45 DAP. Which was found on par with pre-emergence application of metolachlor 50% EC @ 1.0 kg *a.i* ha<sup>-1</sup> followed by hand weeding at 45 DAP for number of flowers per plant (94.00), flower yield per plant (0.53 kg plant<sup>-1</sup>), flower yield per plot (23.20 kg plot<sup>-1</sup>) and flower yield per hectare (17.54 t ha<sup>-1</sup>).

With the best weed control treatments significantly higher number of flowers per plant was recorded

as the crop and weed competition for resources is less and crop acquired more amounts of nutrients and growth is enhanced, that made the crop to branch more and with wider leaf area number of flowers increased in both the seasons compared to unweeded check (Markam *et al.* (2020) in marigold, Gamit *et al.* (2019) in marigold and Jain *et al.* (2015) in tuberose). With respect to radish intercrop growth and flowering recovery can be correlated with similar studies from Thomas *et al.* (2018), in which the contrasting results were obtained for carrot intercrop where, it drastically suppressed main crop during *Rabi* season and failed to control weeds in *Summer* leading to yield loss of both marigold and carrot.

## CONCLUSION

Different weed control treatments significantly influenced the growth of marigold. Significantly, taller plant height, broader plant spread (E-W and N-S), number of primary and secondary branches, leaf area, plant dry weight at grand growth stage and all yield parameters were recorded with weed free treatment which was found to be on par with post-emergent application of imazethapyr (10 SL) @100 g *a.i*/ ha + HW at 45 DAP and pre-emergent application of metolachlor (50 EC) @ 1.0 kg *a.i*/ ha + HW at 45 DAP. Hence, application of imazethapyr (10 SL) @100 g *a.i*/ ha + HW at 45 DAP and metolachlor (50 EC) @ 1.0 kg *a.i*/ ha + HW at 45 DAP can be recommended as better weed management approaches to farmers.

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# Phytochemical Analysis, HPTLC Profile, and *In-vitro* Antioxidant and Antibacterial Activity of *Cyperus rotundus* L. Rhizome Extracts

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## ABSTRACT

*Cyperus rotundus* L. (family: Cyperaceae) is a perennial weed and widely used in traditional medicine in Asian countries. The present study deals with the methanolic, ethanolic, and aqueous extracts obtained from the rhizome part of *Cyperus rotundus* to investigate the preliminary phytochemical analysis, High-Performance Thin Layer Chromatography (HPTLC) profiling, and *in-vitro* antioxidant properties and antibacterial activities against the fish pathogenic bacteria *Edwardsiella tarda* (ATCC 15947). The qualitative phytochemical analysis showed the presence of alkaloids, flavonoids, phenols, terpenoids, tannins, cardiac glycosides, saponins, and steroids in methanolic and ethanolic extracts. Quantitative estimation of the total phenolic and flavonoid contents in methanolic extracts was higher than in other extracts. HPTLC profiling was carried out to visualize the unknown secondary metabolites on densitometry scan at 254 nm, 366 nm 550 nm respectively. In addition, the antioxidant activity was determined by free radical inhibition of the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay. In the result, *in-vitro* antioxidant and antibacterial activities against *E. tarda* (ATCC 15947) exhibited a zone of inhibition of about 12 mm in methanolic extract of *C. rotundus*. Therefore, the current study provides baseline information for exploiting the extract of *C. rotundus* rhizome for treating the *E. tarda* infection in the aquaculture system.

## HIGHLIGHTS

- *Cyperus rotundus* L. rhizome extracts have potential *in-vitro* antioxidant and antibacterial activities against *E. tarda* (ATCC 15947) exhibited a zone of inhibition.

**Keywords:** *Cyperus rotundus*, Phytochemical, HPTLC techniques, *Edwardsiella tarda*, Phytochemical applications

Millions of people throughout the world rely on fisheries and aquaculture as significant sources of food, nutrition, income, and livelihood. With a total production of 14.2 MMT of fish in 2019–2020, India is the second-largest fish producer in the world (Handbook on fisheries statistics 2020). Many infective diseases occur due to bacteria, viruses, fungus, and parasites (Kumari and Sahoo 2006) leading to huge economic losses in the aquaculture system. The most commonly encountered fish-pathogenic bacterial diseases are caused by pathogens such as *Aeromonas hydrophila*,

*Aeromonas salmonicida*, *Vibrio* spp, *Edwardsiella ictaluri*, *E. tarda*, *Streptococcus* spp, and other gram-positive pathogenic bacteria (Pridgeon *et al.* 2012). The systematic bacterial infection caused by *Edwardsiella tarda* is known as Edwardsiellosis (Thune *et al.* 1993).

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Because medicinal plants contain bioactive molecules like alkaloids, terpenoids, saponins, and flavonoids, which have inherent antioxidants, antibacterial, antiviral, and antiparasitic, as well as immunostimulatory properties, herbal medicine is a potent alternative to the use of chemotherapeutic agents and antibiotics, which helps to mitigate the issues related to chemical agents (Pandey *et al.* 2010; Harikrishnan and Balasundaram 2005).

Since many years, *Cyperus rotundus*, a member of the Cyperaceae family, has been used as a traditional medicine in India. Its contents offer antioxidant, anti-inflammatory, antibacterial, antidiabetic, antidiarrheal, and antipyretic properties owing to the presence of essential oils, flavonoids, terpenoids, sesquiterpenes, sitosterol, cyperene, and cyperol; (Nagulendran *et al.* 2007; Singh *et al.* 2012) as well as have antibacterial, anthelmintic, anti-fungal, antiparasitic, and antirheumatic properties (Himaja *et al.* 2014).

Jain *et al.* (2022) revealed with HPTLC technique to identify the occurrence of flavonoids, especially quercetin in the ethanolic extract of *C. rotundus* flower, by using mobile phase toluene-ethyl acetate-formic acid (3:4:2.5 v/v), on a pre-coated plate of silica gel and quantified the amount of quercetin by densitometry absorbance mode at 257 nm. The phenolics and flavonoids are the major groups of secondary metabolites which act as having primary antioxidant properties (Duenas *et al.* 2006). Total oligomeric flavonoid extract of *C. rotundus* has antioxidant, antimutagenic, antigenotoxic, antimicrobial, anticancer, and neuroprotective properties (Kilani *et al.* 2005; Kilani-Jaziri *et al.* 2009). In fact, polyphenolic compounds can play an important role in absorbing and neutralizing the free radicals, quenching singlet and triplet oxygens, or decomposing peroxides (Al-Dabbas *et al.* 2006). In the earlier study Kumar *et al.* (2014) had demonstrated the *in vitro* anti-oxidant and free radical scavenging activities of 70% ethanolic, methanolic and water extracts of rhizome part of *C. rotundus*. The methanolic and aqueous extracts of *Cyperus* rhizome showed considerable antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* at concentrations of 400 and 600 mg/mL.

However, the utility of the rhizome extract of *C. rotundus* for the treatment of any new bacterial

disease in the aquaculture system need to be evaluated in-vitro for assertion. The present study points to evaluate the phytochemical constituents especially in the phenolics and flavonoids contents from the rhizome extracts of *C. rotundus* with further HPTLC fingerprint profiling of extract constituents, and *in vitro* antioxidant, antibacterial activity was tested against the fish pathogenic bacteria *E. tarda*.

## MATERIAL AND METHODS

### Collection of plant and preparation of extracts

The tuber part of *Cyperus rotundus* was collected from agricultural lands and riverine sites and fresh plant roots were rinsed thoroughly with tap water. The root (rhizome) was dried under shade and grained into a coarse powder and stored without moisture. The dried rhizome coarse powder (5 g) was extracted with 50 ml of different solvents such as methanol, ethanol, and aqueous. The extraction process was performed through the Soxhlet apparatus. The extracts were filtered through a Whatman No: 1 filter paper followed by solvent evaporation by a rotary evaporator under reduced pressure to obtain the concentrated extract. The extracts were stored at 4°C in sterilized amber glass bottles until further analysis.

### Preliminary phytochemical analysis

All three extracts of *Cyperus* rhizome were subjected to qualitative tests for the identification of various bioactive constituents following the methods of Devi *et al.* 2014; Gul *et al.* 2017.

**Test for alkaloids:** To the filtrate, Mayer's reagent (**Mayer's reagent:** An amount of 1.36 g of mercuric iodide was dissolved in 60 ml of water-mixed well, containing 5 g of potassium iodide in 20 ml of water) was added, and a cream precipitate indicates the presence of alkaloids. Measured 2 ml of various extract solutions were then mixed with dilute hydrochloric acid and 0.1 ml of Wagner's reagent. The formation of a reddish-brown colour precipitate indicates a positive response for alkaloids.

**Test for glycosides:** To 1 ml of all the plant extracts, equal volumes of Fehling's solution A and B (**Fehling's solution A:** A weight of 34.64 g copper sulfate was dissolved in 0.5 ml of sulphuric acid and sufficient water was added to make up to 500 ml volume. **Fehling's solution B:** A weight of 176 g





of sodium potassium tartrate and 77 g of NaOH is dissolved in a sufficient volume of water to produce 500 ml) were added. The mixture was heated in a water bath. Brick red coloration indicated the presence of glycosides.

**Tests for flavonoids (Alkaline Reagent Test):** Two ml of 2.0% NaOH mixture was mixed with aqueous extract of the plant rhizome. An intense yellow colour produced, which becomes colourless when added two drops of diluted acid to the mixture. This result shows the presence of flavonoids.

**Test for phenols (Ferric chloride test):** Around 5 ml of the extract solution was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicates the presence of tannins.

**Test for saponins:** About 10 ml of the extract was mixed with 5 ml of distilled water and shaken vigorously; formation of a stable persistent-froth indicates the presence of saponins.

**Test for steroids (Salkowski test):** Concentrated sulphuric acid was added to 10 mg of different extracts dissolved in 1 ml of chloroform. A reddish-blue colour exhibited by the chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

**Test for tannins:** Around 5 ml of extract solutions was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black coloration indicates the presence of tannins.

**Test for terpenoids:** About 5 ml of the extract was treated with 2 ml of chloroform and the concentrated sulphuric acid was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

### Estimation of total phenolics and flavonoids content

#### Total phenolic Assay

The total phenolic contents were determined using the Folin-Ciocalteu assay (Singleton *et al.* 1965). An aliquot (1 ml) of extract or standard solution of Gallic acid (20, 40, 60, 80, and 100 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. Reagent blank using distilled water was also prepared. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes, 10 ml of 7% Na<sub>2</sub>CO<sub>3</sub> solution was added

to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with a UV-Visible spectrophotometer. Total phenolic content was expressed as mg Gallic acid equivalents (GAE).

#### Total Flavonoid Assay

Total flavonoid content was measured by the aluminium chloride colorimetric assay (Chang *et al.* 2002). An aliquot (1 ml) of extract or standard solution of quercetin (20, 40, 60, 80, and 100 µg/ml) was added to a 10 ml volumetric flask containing 4ml of distilled water. To the flask 0.30 ml, 5% NaNO<sub>2</sub> was added following the addition of 0.3 ml 10% AlCl<sub>3</sub> after 5 minutes. After five minutes, 2 ml 1M NaOH was added to make up 10 ml with distilled water. The solution was mixed, and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

#### Antioxidant DPPH scavenging activity

The antioxidant activity of different extracts was determined by DPPH radical scavenging assay (Tepe *et al.* 2005). The hydrogen atoms or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable 2,2'-diphenylpicrylhydrazyl (DPPH) radical as a reagent.

One ml of various concentrations of the extracts in methanol was added to 1 ml of a 0.004% methanol solution of DPPH. After 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (I%) was calculated in following way:

$$\text{DPPH scavenging activity (I\%)} = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100$$

Where,

$A_{\text{blank}}$  is the absorbance of the control reaction (containing all reagents except the test compound), and  $A_{\text{sample}}$  is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC<sub>50</sub>) was calculated from the graph plotted



with the inhibition percentage against extract concentration. Tests were carried out in triplicate.

### HPTLC (High-Performance Thin Layer Chromatography) profile

HPTLC is commonly used to produce a plant's chemical fingerprint to identify and quantify the main constituent within the plant. HPTLC analysis was performed using the CAMAG HPTLC system (Switzerland). Samples of 10 $\mu$ l were applied to an aluminium-backed, pre-coated silica gel 60F254 TLC plate (0.2 mm thickness) (Merck India) (size: 5.0  $\times$  10.0 cm) as bands at 10 mm from the bottom of the plate and 15 mm from the sides using CAMAG ATS 4.

The chromatogram was developed with different mobile phase such as:

- ♦ Toluene: Ethyl acetate: methanol (5:5:1) for Methanolic and Ethanolic extract of *C. rotundus*.
- ♦ Toluene: Ethyl acetate: methanol: formic acid: water (9:1:0.5:0.5) for Water extract of *C. rotundus*.

The chromatogram was developed in a saturated Twine trough chromatographic chamber (Camag, Switzerland). The designed plate was visualized under UV at 254 nm and 366 nm and in visible light after derivatizing with Anisaldehyde sulphuric acid reagent followed by heating at 105°C for 5 minutes. Densitometric scanning was done with TLC Scanner 3 at 254 nm, 366 nm, and 550 nm respectively.

### Bacterial Strain

*In vitro* antimicrobial activity was examined for methanol, ethanol, ethyl acetate, and aqueous extracts from the rhizome part of *C. rotundus*. *E. tarda* used in the study was obtained from ATCC 15947, USA. *E. tarda* (ATCC 15947) was revived using Brain heart infusion (BHI) broth from the culture loop. The broth was incubated for 18-24 hours at 37°C. The bacterial culture was then streaked on Salmonella-Shigella (SS) Agar media and incubated at 28°C. The microorganism's culture was maintained at 4°C on BHI nutrient agar plate.

**Enhancing virulence of the bacterial strain:** Fresh *E. tarda* culture in 5 mL BHI broth grown at 28°C was centrifuged, and the pellet was dissolved in 5 mL of PBS solution. From this 0.1 mL was injected into a naïve rohu fish, and the animal

was sacrificed after 12 h. *E. tarda* bacteria were re-isolated from the injected fish's kidney, streaked in the SS agar plate, and incubated for 16 h at 28°C.

The next day one single black colony was inoculated in 5mL of BHI broth and incubated for 12-16 h at 28°C. Again, one naïve fish was injected, and in the same manner, re-isolation was done, and the bacterial culture was utilized for estimation of LD<sub>50</sub> and challenge study.

**Isolation & Morphological identification of *Edwardsiella tarda*:** Tissue samples from the kidney, and liver of *Labeo rohita* were taken by sterile loop and streaked on SSA plate (Himedia) and incubated at 37°C for 24 hours. Black centric colonies showing or resembling with morphological characteristics of *E. tarda* were further sub-cultured on BHT brain heart infusion agar plates and incubated at 37°C for 24 hours. Presumptive identification of the resulting isolates (colonies) was done employing direct tests which included primary bacteria identification techniques and biochemical identification tests.

**Preparation of McFarland Standard and bacterial inoculum:** McFarland number 0.5 standard was prepared by mixing 9.95 ml of 1% H<sub>2</sub>SO<sub>4</sub> and 0.05 ml 1% BaCl<sub>2</sub> in distilled water to estimate bacterial density. The preparation was stored in an airtight bottle and used for comparison of bacterial suspension whenever required. Bacterial isolates were inoculated in 5ml sterile nutrient broth tubes and incubated overnight at 30°C. Turbidity of the cultures were compared with 0.5 MacFarland standard and adjusted by adding sterile normal saline or more bacteria.

The antibacterial assay was performed by two methods viz. and the agar well diffusion method (Perez *et al.* 1990).

### Agar well diffusion method

The molten Mueller Hinton Agar plates were inoculated with the 100  $\mu$ l of bacterial strain ( $1 \times 10^8$  CFU) under aseptic conditions and the developed wells (6mm diameter) were filled with 100  $\mu$ l of the test extract samples (Concentration 5, 2.5, and 1.25 mg/mL) and 100  $\mu$ l of DMSO (100%) & Amoxicillin (30 mg/mL) were used as controls. The plate was incubated at 37 °C for 24 hours. After incubation, the result of bacterial growth was determined by measuring the diameter of the zone of inhibition (mm). Tests were performed in duplicate.



## Statistical analysis

All data were presented as means  $\pm$  S.D. Statistical analysis for all the assays results was done using the Microsoft Excel program.

## RESULTS

### Preliminary phytochemical screening

The present study carried out on the rhizome of *Cyperus rotundus* revealed the presence of active phytochemical constituents. The methanol, ethanolic and aqueous extracts from the rhizome tuber showed positive results for tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, steroids, and phenolic compounds as measured by the different test procedures presented in (Table 1). Therefore, the present study indicates a wide range of phytochemicals availability in the *Cyperus rotundus* rhizome extract.

**Table 1:** Phytochemical screening of bioactive metabolites from rhizome part of *C. rotundus* L. extracts

Test compounds	CR Methanol extract	CR Ethanol extract	CR Aqueous extract
Alkaloids (Mager's test)	-	+	+
Glycosides (Fehling's solution test)	+	-	-
Phenols (Alkaline test)	++	+	++
Flavonoids (Ferric chloride test)	++	++	+
Saponin (Foam test)	+	++	++
Steroids (Salkowski test)	++	+	-
Tannins (Ferric chloride test)	+	+	-
Terpenoids (Chloroform test)	++	+	-
Quinones	++	+	-

Key: "+" Presence; "-" Absence.

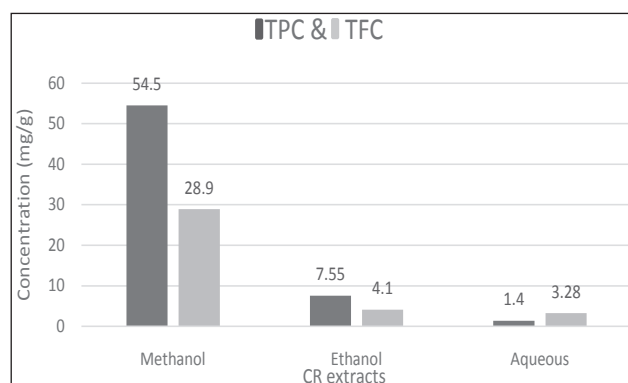
### Estimation of total Phenolics and Flavonoids content

Total phenolic and flavonoid compounds were recorded in different concentrations in *C. rotundus* rhizome extract. The test result of total phenolic and flavonoids contents in methanol, ethanol,

and aqueous extract of *C. rotundus* rhizome show significantly different profiles as shown in Fig. 1 with the highest amount in the methanolic extract (Table 2); (Fig. 1),

**Table 2:** Total phenolic content (mg Gallic acid equivalent phenol/g) and total flavonoid content (mg quercetin equivalent flavonoid/g) of *Cyperus rotundus* L. rhizome extracts

CR rhizome Extracts	TPC mg gallic acid eqv / g of sample $\pm$ SD	TFC mg quercetin eqv / g of sample $\pm$ SD
Methanol	54.5 $\pm$ 3.21	28.9 $\pm$ 0
Ethanol	7.55 $\pm$ 1.91	4.1 $\pm$ 0.14
Aqueous	1.4 $\pm$ 0.11	3.28 $\pm$ 0.34



**Fig. 1:** Total phenolic content (mg Gallic acid equivalent phenol/g) and total flavonoid content (mg quercetin equivalent flavonoid/g) of *Cyperus rotundus* L. rhizome extracts

### HPTLC fingerprint profile

The results from HPTLC fingerprint scanned at different wavelengths of 256 nm, 366 nm, 550 nm using the different solvent system and derivatization reagents (Table 3) for methanolic, ethanolic and aqueous extract of *Cyperus rotundus* (CR) rhizome confirmed the presence of other many secondary metabolites.

### Methanolic extract of *Cyperus rotundus* L. (CR)

The HPTLC densitometry scan of methanol extract of CR rhizomes showed different polyvalent phytoconstituents. The ascending order of R<sub>f</sub> values 0.78 (area maxima - 8.91%), 0.46 (area maxima- 27.98%) and 0.74 (area maxima- 33.4%) at 254 nm, 366nm 550 nm respectively were observed (Table 4 & 5).

**Table 3:** HPTLC-fingerprint profiling solvent system.

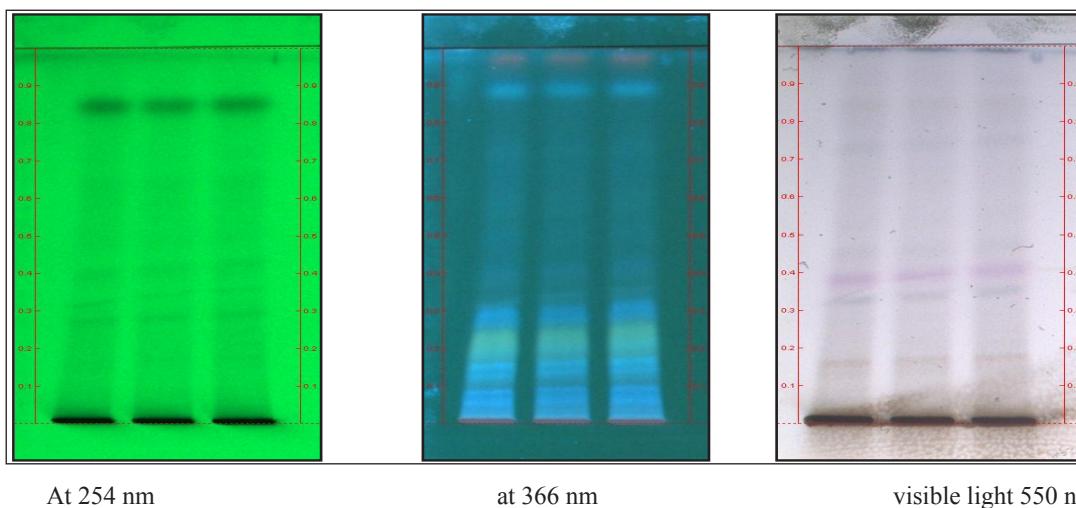
<i>Cyperus rotundus</i> L. Rhizome part extracts	Solvent system used	Volume ratio (v/v)	Post derivation Reagents used
Methanol Extract	Toluene: Ethyl acetate: Methanol	5:5:1	Anisaldehyde Sulphuric acid
(70%) Ethanol Extract	Toluene: Ethyl Acetate: Methanol	5:5:1	Anisaldehyde Sulphuric acid
Aqueous Extract	Ethyl Acetate: Methanol: Formic acid: water	9:1:0.5:0.5	Anisaldehyde Sulphuric acid

**Table 4:** HPTLC profiling on maximum Rf values of methanolic extract of *C. rotundus*

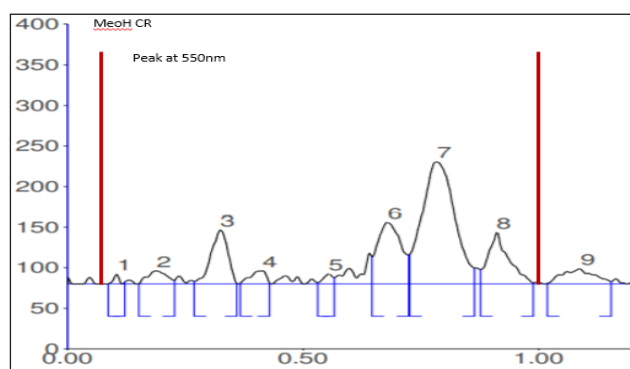
Sample	254 nm		366 nm		Post-Derivatize plate 550 nm	
	Rf	Colour of bands	Rf	Colour of bands	Rf	Colour of bands
Methanolic extract	0.17	Dark	0.06	Light blue	0.19	Light black
	0.28	Dark	0.09	Light blue	0.33	Light black
	0.36	Dark	0.15	Light blue	0.41	Light pink
	0.43	Dark	0.17	Light blue	0.56	Light black
	0.64	Dark	0.23	Light yellow	0.68	Light black
	0.78	Dark	0.30	Light blue	0.79	Light black
			0.46	Light blue	0.91	Light black
			0.89	Light blue		
		0.97	Light red			

**Table 5:** The post derivatization HPTLC profile under visible light at 550 nm of the methanolic extract of *Cyperus rotundus* L.

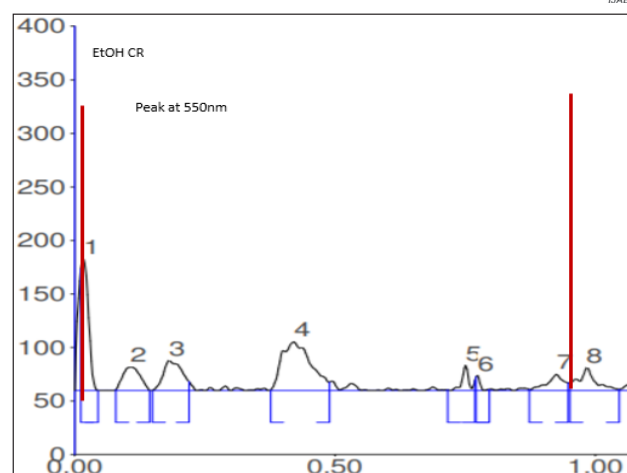
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.09	0.4	0.11	12	1.53	0.12	2.5	63.7	0.35	unknown
2	0.15	0.4	0.19	16.3	2.07	0.23	4.5	311.8	1.73	unknown
3	0.27	3.2	0.33	66.6	8.45	0.36	0.9	1112.7	6.17	unknown
4	0.37	0.8	0.41	16.3	2.07	0.43	1.2	257.2	1.42	unknown
5	0.53	1.8	0.56	12.6	1.59	0.57	7.8	117.9	0.65	unknown
6	0.65	33.4	0.68	75.9	9.63	0.72	35.7	1790.2	9.92	unknown
7	0.73	36.3	0.79	150.4	19.08	0.86	19.5	4864.5	26.96	unknown
8	0.88	17.9	0.91	63.3	8.04	0.99	1.1	1280.3	7.09	unknown



**Fig. 2:** Methanolic extract CR rhizome in HPTLC plate visualized at various wavelengths for coloured bands



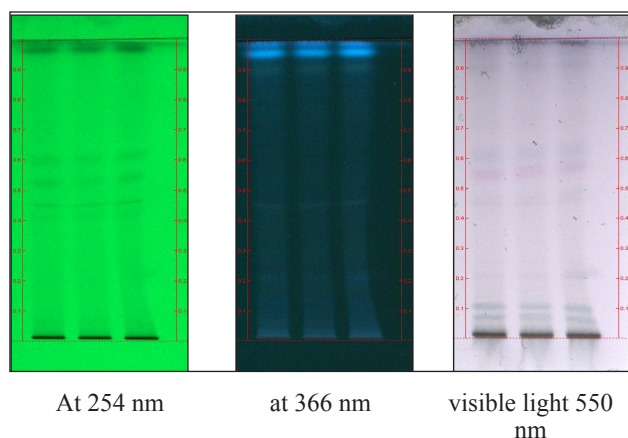
**Fig. 3:** Post derivatization HPTLC chromatogram of Methanolic extract of *Cyperus rotundus* L. rhizome



**Fig. 5:** Post derivatization HPTLC chromatogram of Ethanolic extract of *Cyperus rotundus* rhizome

### Ethanolic extract of *Cyperus rotundus* L. (CR)

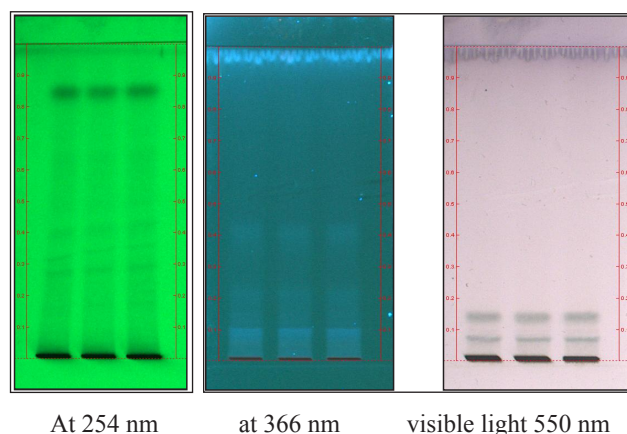
In HPTLC scanning result for ethanol extract of CR rhizomes showed different polyvalent phytoconstituents. The descending order of Rf values 0.89 (area maxima - 10.71%), 0.42 (area maxima- 16.25 %) and 0.18 (area maxima- 7.69 %) at 254 nm, 366 nm 550 nm respectively were observed (Table 6 & 7).



**Fig. 4:** Ethanolic extract of CR rhizome in HPTLC plate visualized at various wavelengths for coloured bands

### Aqueous extract of *Cyperus rotundus* (CR)

The HPTLC densitometry scan for aqueous extract of CR rhizomes showed different polyvalent phytoconstituents. The ascending order of Rf values 0.17 (area maxima - 4.96%), 0.18 (area maxima- 8.41%) and 0.26 (area maxima- 31.59%) at 254 nm, 366 nm 550 nm respectively were observed (Table 9 & 10).



**Fig. 6:** Aqueous extract of CR rhizome in HPTLC plate visualized at various wavelengths for coloured bands

**Table 6:** HPTLC profiling on maximum Rf values of ethanolic Extract of *C. rotundus* L.

Sample	254 nm		366 nm		Post-Derivatize plate 550 nm	
	Rf	Colour of bands	Rf	Colour of bands	Rf	Colour of bands
Ethanol extract	0.41	Dark	0.21	Very light blue	0.02	Light dark
	0.45	Dark	0.42	Very light blue	0.11	Light dark
	0.53	Dark	0.90	Very light blue	0.18	Light dark
	0.61	Dark	0.96	Light blue	0.42	Light dark
	0.89	Dark			0.75	Light pink
	0.90	Dark			0.78	Light dark
					0.93	Light dark

**Table 7:** The post derivatization HPTLC profile of the Ethanolic extract of *Cyperus rotundus* L: at visible light 550 nm

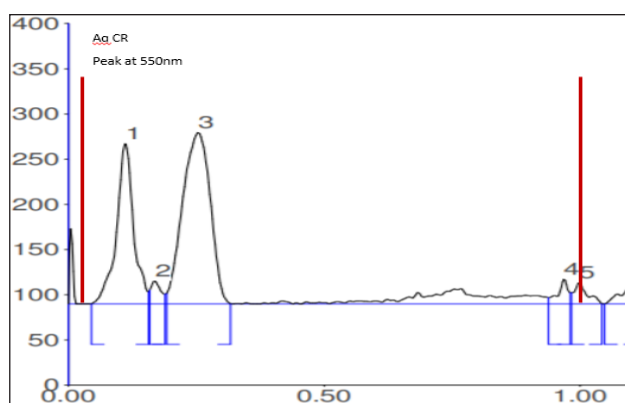
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.01	113.8	0.02	123.2	32.95	0.04	0.1	841.6	13.89	unknown
2	0.08	0.7	0.11	22	5.88	0.14	0.2	345.4	5.7	unknown
3	0.15	0.1	0.18	28.3	7.57	0.22	8.6	466.2	7.69	unknown
4	0.38	0.7	0.42	45.4	12.15	0.49	8	1202.8	19.85	unknown
5	0.72	0.7	0.75	23.5	6.28	0.77	9.6	146.7	2.42	unknown
6	0.77	13.2	0.78	14.2	3.79	0.8	1.4	54	0.89	unknown
7	0.88	1.1	0.93	15	4.02	0.95	7.3	228	3.76	unknown

**Table 8:** HPTLC profiling on maximum Rf Values of Aqueous Extract of *C. rotundus* L. rhizome

Sample	254 nm		366 nm		Post-Derivatize plate 550 nm	
	Rf	Colour of bands	Rf	Colour of bands	Rf	Colour of bands
Aqueous Extract	0.06	Very light dark	0.11	Very light blue	0.07	light Dark
	0.17	Very light dark	0.17	Very light blue	0.12	Light dark
	0.75	Light dark	0.26	Very light blue	0.26	Light dark
	0.91	Light dark	0.97	Very light blue	0.91	Light dark

**Table 9:** The post derivatization HPTLC profile of the Aqueous extract of *Cyperus rotundus* L. rhizome: at visible light 550nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.04	0	0.11	177.5	25.62	0.16	13.3	2760.2	16.14	unknown
2	0.16	15.7	0.17	25.4	3.66	0.19	10.9	236.1	1.38	unknown
3	0.19	11.8	0.26	189.8	27.39	0.32	0.32	4787.4	27.99	unknown
4	0.94	7.3	0.97	27.6	3.98	0.98	0.98	250.2	1.46	unknown



**Fig. 7:** Post derivatization HPTLC chromatogram of Aqueous extract of *Cyperus rotundus* L. rhizome

### Antioxidant -DPPH assay

The rhizome of *C. rotundus* methanolic, ethanolic, and aqueous extracts were subjected to characterize their possibility of antioxidant properties by DPPH assay; the results are expressed in the percentage of free radical inhibition. The reaction

is only dependent on extracts containing chemical constituents. The free radical scavenging activity of methanolic extracts has a higher % inhibition than other extracts shown in table 10.

**Table 10:** DPPH scavenging activity of *C. rotundus* extracts

Standard/ Extracts	DPPH Inhibition activity %
Quercetin (standard)	38.3
Methanol extract	50.63
Ethanol extract	48.62
Aqueous extract	48.45

### In vitro antibacterial activity

#### Identification of test bacteria strain

*Edwardsiella tarda* strain (ATCC 15947) inoculum was injected to the fingerling of *Labeo rohita* and re-isolated from the infection sites to confirm with the biochemical test for *E. trada* isolate.

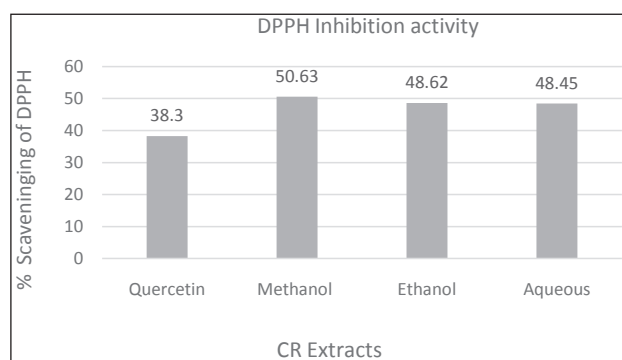


Fig. 8

### *In vitro* antibacterial activity of CR rhizome extracts

*In vitro* antibacterial assay was estimated by the agar well diffusion method. Amoxicillin was used as a positive control. The methanolic extract of CR rhizome showed a zone of inhibition superior followed by the ethanolic extract. The zone of inhibition of methanolic extract exhibited a zone length of 8-12 mm against *E. tarda* (ATCC 15947). Its inhibition activity depends on various concentrations. But, the ethanolic extract showed a moreover similar inhibition zone of 10-11 mm against *E. tarda*. However, no inhibition was observed in aqueous extracts of the rhizome of *Cyperus rotundus*. The results of the zone of inhibition activity pattern of bacterial test isolates are presented in Table 11.

**Table 11:** *In vitro* antibacterial activity of CR rhizome extracts against *E. tarda* (ATCC 15947)

<i>C. rotundus</i> Extracts (Concentration)	Antibacterial activity (Zone of Inhibition diameter (mm))		
	5 mg/ml	2.5 mg/ml	1.25 mg/ml
Methanol extract	12 ±1.41	11.5 ±0.71	8 ±0.1
Ethanol extract	11 ±0.71	10.5 ±0.71	0
Aqueous extract	0	0	0
Amoxicillin (30 mg/ml)	Zone of Inhibition (mm)- 38 ± 0.14		

## DISCUSSION

### Preliminary phytochemical screening

The secondary bioactive metabolites contained in the rhizome of *C. rotundus* extracts are made known by various types of phytoconstituents test in the methanolic, ethanolic, and aqueous extracts. In the

methanolic extract, all the tested phytoconstituents were obtained except alkaloids. Further, the ethanolic extract of the rhizome could show all the compounds tested excluding glycosides. However, only a few classes of compounds were observed in aqueous extract such as alkaloids, phenols, flavonoids, and saponin. The bioactive molecules of phenols, flavonoids, tannins, alkaloids, terpenoids, steroids and polypeptides have showed antiparasitic, antifungal, antibacterial, anti-inflammatory, antiviral and immunostimulant properties (Citarasu 2010; Pandey *et al.* 2012; Nair *et al.* 2019).

### Total phenolics and flavonoids content

The phenolic and flavonoids compounds have redox properties that can inhibit the activity of many enzymes such as xanthine oxidase, peroxidase, and nitric oxide synthase, which actively participate in the free radical generation, thereby resulting in decreased oxidative damage of macromolecules (Cazarolli *et al.* 2008). The phenolic content was measured using the Folin-Ciocalteu reagent in each extract. The obtained different concentrations of total phenolic compounds (TPC) were  $54.5 \pm 3.21$ ,  $7.55 \pm 0$ , and  $1.4 \pm 0.11$  mg Gallic acid equivalent/g for methanol, ethanol, and aqueous extracts of *C. rotundus* rhizome, respectively. The highest phenolics content was observed in methanolic extracts of *C. rotundus* rhizome followed by other extracts. A similar trend was observed in the study of Kumar *et al.* (2014) where in a total phenol content (TPC) of  $254.50 \pm 5.26$  µg GAE/mg dry weight in the 70% ethanol extract and  $192.77 \pm 5.48$  µg GAE/mg dry weight in the methanol extract and  $70.75 \pm 4.48$  µg GAE/mg dry weight in the water extract of *Cyperus rotundus* L was found.

Flavonoids are secondary metabolites which have the potential antioxidant properties depending on the number and position of free OH groups. The total flavonoid content of different extracts of methanol, ethanol, and aqueous were estimated spectrophotometrically. The highest flavonoid content was observed in the methanolic extracts of *C. rotundus* rhizome followed by other extracts. Total flavonoids content (TPC) on methanolic, ethanolic, and aqueous extracts are found as  $28.9 \pm 0$ mg,  $4.1 \pm 0.14$  mg, and  $3.28 \pm 0.34$  mg quercetin equivalent /g sample extract of *C. rotundus* L, respectively. In contrast, Kumar *et al.* (2014) reported



a total flavonoid content (TFC) of  $164.34 \pm 3.75 \mu\text{g CE/mg}$  dry weight in the 70% ethanol extract and  $138.01 \pm 5.24 \mu\text{g CE (catechin)/mg}$  dry weight in the methanol extract and  $51.23 \pm 2.62 \mu\text{g CE/mg}$  dry weight in the water extract of *Cyperus rotundus* L.

### High Performance Thin Layer Chromatography (HPTLC) profile

According to the HPTLC profile, each solvent extract of *C. rotundus* rhizome has shown various bands representing a separate class of bioactive compounds. HPTLC fingerprint technique is based on TLC, which increases the high resolution of secondary phytochemical constituents to be separated on the TLC plate (Attimarad *et al.* 2011).

Different colours of band exhibited after derivatization with p-anisaldehyde/ sulfuric acid reagent was used for visualization at different wavelengths at 254 nm, 366 nm, and 550 nm were seen as dark, light blue zones representing a wide variety of secondary metabolites present in the extracts. The methanolic, ethanolic, and aqueous extracts of *Cyperus* rhizome showed especially superior separation in Toluene: Ethyl acetate: Methanol (5:5:1) solvent system of methanol extract. The methanol extract of *Cyperus rotundus* rhizome showed different polyvalent phytoconstituents (Corresponding to the number of band separations). In HPTLC densitometric scan at 550 nm for methanol extract the maximum Rf value 0.79, found to be predominant as the percentage area, 33.4%.

### In vitro antioxidant assay

Plant extracts are responsible for facilitating free radical scavenging activity which depends on the presence of the free OH group. The hydroxyl groups in phenolic and flavonoid compounds have the potential antioxidant capability via exhibiting free radical inhibition, peroxide decomposition, metal inactivation, or oxygen scavenging in biological systems that are implicated in preventing oxidative disease (Babbar *et al.* 2015; Agati *et al.* 2012; Sooratte *et al.* 2005). DPPH assay is a rapid and sensitive method to detect the scavenging ability of the plant extracts due to their hydrogen donation properties (Philips *et al.* 2010). Aqueous and ethanolic extracts of *C. rotundus* revealed high free radical scavenging activities in a dose-dependent manner up to  $80 \mu\text{g/}$

ml and also a dose-independent manner (Soumaya *et al.* 2014).

The present study shows that the in-vitro antioxidant activity of *C. rotundus* rhizome extracts has free radical scavenging capacity with a higher percentage of inhibition in the methanolic extracts followed by ethanolic and aqueous extracts.

### In vitro antibacterial test

Karzan *et al.* (2017) reported that both the aqueous and ethanolic extracts of *Cyperus* rhizomes-tubers showed antibacterial activity against *E. coli* and *S. aureus*. The observed variation in results with the present study might be due to the varied sensitivities of microorganisms. The polyphenolic compounds can form highly soluble complexes with proteins. They can bind to bacterial adhesions and disturb the availability of receptors on the cell surface (Haslam 1996). Tannins have been shown to form irreversible complexes with proline-rich proteins (Hagerman and Butler 1981), resulting in inhibition of cell wall protein synthesis.

The previous report states that the limited hydrophobic nature of the phenolic compounds may be responsible for their antimicrobial action. Further, the inhibition of hydrolytic enzymes (proteases) or other interactions inactivates microbial adhesins, cell envelope transport proteins, and non-specific interactions with carbohydrates (Pyla *et al.* 2010).

The *C. rotundus* rhizome showed the in vitro antibacterial activity against fish pathogenic bacteria *E.tarda* in methanolic and ethanolic extracts with the formation of zone of inhibition at the lower contraction of extracts 5 mg/ml, 2.5 mg/ml. But, there is no antibacterial activity in the aqueous extracts.

### CONCLUSION

In the present study, *C. rotundus* rhizome extracts exhibit both in-vitro antioxidant, and antibacterial activity due to the presence of known and unknown phytoconstituents. Further studies are required to identify the other bioactive potential compounds in different solvent extraction methods. The rhizome of *C. rotundus* has medicinal properties that may be used for drug development in the future and has application as herbal products for the bio-control of diseases, as a novel emerging alternative





for antimicrobial treatment leading to nontoxic and more environmentally managing of virulent diseases. Hence, this study provided evidence that *Cyperus rotundus* extract can be used in the aquaculture systems against pathogenic bacteria, *E. tarda*.

## ACKNOWLEDGMENTS

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# Cryobiotechnological Tool: Cryopreservation of *in vitro* Grown Shoot tips of Grape (*Vitis vinifera* L.) cv. Fantasy Seedless

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## ABSTRACT

Cryotherapy is a relatively new application of plant cryopreservation techniques that consists in a promising tool, coupled with meristem culture, for achieving in a short time, high frequency of regenerating plants free of viruses. Cryoprotectants should be prevention of intracellular ice crystal formation, should be biologically acceptable, must be able to penetrate into the cells and should be less toxic. Vitrification, encapsulation – dehydration, encapsulation – vitrification and droplet vitrification are approaches to achieve a successful cryopreservation. Cryotherapy based procedures can be easily implemented in healthy plant production and long term germplasm conservation. This technique facilitates the treatment of large numbers of samples, result in remarkable frequencies of pathogen-free plants and prevent the difficulties associated with the excision of small shoot tips. Axillary shoot tips of *in vitro* grape (*Vitis vinifera* L. cv. Fantasy Seedless) were successfully cryo-preserved by vitrification. Axillary shoot tips were excised from 5 month old plantlets and were cultured on solidified MS medium. Shoot tips were pre cultured on 0.3 M sucrose for 3 days and then treated with dehydrated with PVS2 for 60 min at 0 °C before plunged into liquid nitrogen for 1 hr. Samples were then warmed rapidly in water. The recovery of shoot tips 70 per cent. Half strength PVS2 solution was used to improve the further recovery and long term storage of shoot tips.

## HIGHLIGHTS

- ❶ Cryotherapy is a relatively new application of plant cryopreservation techniques that consists in a promising tool.
- ❷ Axillary shoot tips of *in vitro* grape (*Vitis vinifera* L. cv. Fantasy Seedless) were successfully cryo-preserved by vitrification
- ❸ Half strength PVS2 solution was used to improve the further recovery and long term storage of shoot tips.

**Keywords:** Cryotherapy, Cryo-protectant, Vitrification, Encapsulation, Cryopreservation and shoot tip

Cryotherapy is a relatively new application of plant cryopreservation techniques that consists in a promising tool, coupled with meristem culture, for achieving in a short time, high frequency of regenerating plants free of viruses (Engelmann, 2004). *Cryo* is Greek word (*krayos* – frost) It literally means preservation in “frozen state”. Basic principle of the technique involves “To bring plant cells or tissue to a zero metabolism and non dividing state

by reducing the temperature in the presence of cryoprotectant”. Grapevine (*Vitis* spp.) is one of the most economically important temperate fruit

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crops. More emphasis is given for exploring the high-valued *Vitis* cultivars and wild species in grape breeding, which may be maintained within genebanks. Shoot tip is most feasible explant that can be used in breeding because of higher rate of meristematic cells avoid pathogen infections. Shoot tip cryopreservation is a valuable technique for the safe, long-term conservation of *Vitis* genetic resources that complements traditional field and *in vitro* germplasm collections. Virus-free plants of cv. Fantasy seedless are required as propagation material for clonally propagated germplasm, and also for the global exchange of genetic resources because of its attractive colour and size. Shoot tip cryotherapy of this cultivar, a method based on cryopreservation, has proven to be effective in eradicating viruses from infected plants. The present study carried out to observe the advances in shoot tip cryopreservation and cryotherapy that have resulted in healthy plants with high re-growth levels.

## MATERIALS AND METHODS

The plant material employed for this study were consisted of *in vitro* plants of Fantasy grapes these cultures were established at tissue culture laboratory, University of Horticultural Sciences, Bagalkot.

### Methods

Collection and preparation of explants: Healthy explants were collected from grape orchard maintained at fruit science block 70 sector Navanagar. Before inoculation, surface sterilization of explants is necessary. Twigs containing shoot tip as well as 3-4 nodes were taken from the current season shoots of mature tree. The explants were washed thoroughly in running tap water to remove debris. They were further washed 3-4 times with distilled water containing few drops of antiseptic (Tween-20) solution. Further explants washed 5-6 times under running tap water in order to remove the adhered solution completely. The explants were cut into shoot tip of (1 cm), then washed repeatedly 4-5 times with sterile water under laminar air flow cabinet. After washing, explants were dipped in fungicide (Dithane M-45, 1.00 g/l) + Bactericide (Streptomycin, 500 mg/l) solution for 15-20 minute then explants were washed with sterile distilled

water for 4-5 times. Further treated with another sterilant *viz.*, citrimide (500 mg/l) for 15-20 minute again washed with sterile distilled water for 6-7 times. Surface sterilization with mercuric chloride ( $\text{HgCl}_2$ ) of 0.01 % for 3 minute.

Inoculation : Surface sterilized explants (Shoot tips) were cultured on establishment (initiation) medium (MS B + BAP 1.00 mg/l + NAA 0.25 mg/l) and aspect culture was raised under standard culture condition of temperature, relative humidity and light. After establishment, initiated or established micro shoots were transferred to growth rooms (lights off 5 °C to 60 °C Lights on 10 °C to 60 °C with humidity of 50-90 %). Stored cultures were sub-cultured every 40-50 days. Plantlets derived from culture were then grown with 75 ml medium in a 200-ml mericlone flask under white fluorescent light ( $50 \mu \text{mol s}^{-1} \text{m}^{-2}$ ) with a photoperiod of 16 hr at 25 °C.

### Cryogenic procedure

Excised shoot tips were pre-cultured on solidified 1/2 MS medium supplemented with different concentrations of sucrose (0.1 to 0.7 M) for 1 or 3 days at 25 °C. Pre-cultured shoot tips were then treated with a mixture of 2 M glycerol plus 0.4 M sucrose in MS medium (LS solution, Nishizawa *et al.* 1992) for 20 min at 25 °C before being dehydrated with a highly concentrated vitrification solution (PVS2) (Sakai *et al.* 1990). PVS2 contains 30% (w/v) glycerol, 15% (w/v) EG, 15% (w/v) DMSO and 0.4 M sucrose in MS medium at pH 5.8. Shoot tips were dehydrated with PVS2 at 0 °C for 80 min (one-step dehydration) or dehydrated with a 50% PVS2 (half-strength solution), followed by full strength PVS2 for 50 min at 0 °C (two-step dehydration). Sufficiently dehydrated shoot tips were placed in a 2.0 ml cryotube containing 1 ml PVS2 and then plunged into LN and held for at least 60 min. One-step vitrification procedure Precultured and osmoprotected shoot tips were placed in a 2.0 ml cryotube and directly dehydrated with 1.5 ml of PVS2 at 0 °C for different lengths of time. Solution was removed and replaced 1.0 ml of fresh PVS2. Cryotubes were plunged into LN and held for at least 60 min.

Two-step vitrification procedure Precultured and osmoprotected shoot tips were first dehydrated with a 50% (half-strength) PVS2 solution, which consisted of 15% (w/v) glycerol, 7.5% (w/v) EG, 7.5% (w/v)

DMSO and 0.4M sucrose in MS medium at pH 5.8, for 30 min at 0 °C. Samples were dehydrated with full strength PVS2 for 50 min at 0 °C. Warming and dilution procedure Cryotubes were rapidly warmed in water at 40 °C for 1 min. PVS2 was drained and replaced with 1.2 m sucrose in MS medium and incubated for 20 min. Shoot tips were then transferred onto sterilized filter paper discs over a recovery medium (solidified 1/2 MS basal medium containing 1 mg l<sup>-1</sup> BA, 3% sucrose and 0.2% gellan gum). After one day, the shoot tips were transferred onto a fresh paper disc in a Petri dish containing the same medium.

## RESULTS AND DISCUSSION

To enhance osmotolerance to PVS2, excised shoot tips were precultured with different concentrations of sucrose for 1 or 3 days. Precultured shoot tips were then treated with a mixture of 2 M glycerol plus 0.4 M sucrose (LS) for 20 min before being dehydrated with PVS2. Shoot tips precultured with 0.3 M sucrose for 3 days, followed by treatment with LS for 20 min produced the highest recovery after dehydration with PVS2 with and without plunging into LN (Table 1). As shown in Table 2, neither the preculturing of explants with 0.3 M sucrose nor treatment with LS solution provided recovery after cryopreservation. However, shoot tips precultured with 0.3 M sucrose for 3 days, followed by treatment with LS solution produced 70% recovery after plunging into LN. A similar tendency on recovery of shoot tips was observed in the samples without plunging into LN.

To increase further recovery after cryopreservation, a two-step dehydration procedure was examined as an alternative. Osmoprotected shoot tips were previously dehydrated with a 50% PVS2 for 30 min at 0 °C, followed by full-strength PVS2 for 50 min at 0 °C before being plunged into LN. This two-step dehydration gave a higher recovery was observed over that of the one-step procedure (Table 3). Vitrified and rewarmed shoot tips turned brown after plating, but resumed growth within 20 days and developed shoots following an additional 80 days without intermediary callus formation. The rooting of elongated shoots was achieved by transferring to solidified hormone free 1/2 MS medium. No morphological abnormalities were observed in the plants developed from cryopreserved shoot tips by vitrification.

Vitrification requires a highly concentrated solution, which sufficiently dehydrates tissues without causing injury, enabling them to form a stable glass along with the surrounding PVS2 when plunged into LN. Thus, the acquisition of osmotolerance for shoot tips to PVS2 is essential in obtaining successful cryopreservation by vitrification. In the shoot tips of grape, preculture with sucrose alone did not produce a high level of recovery growth. In this study, induction of osmotolerance to PVS2 was achieved by preculturing excised shoot tips with 0.3 M sucrose for 3 days, followed by a mixture of 2 M glycerol plus 0.4 M sucrose (LS) for 20 min. The same positive effects of LS treatment following sucrose preculture have repeatedly been reported in temperate and tropical plants (Matsumoto *et al.* 1994; Thin *et al.* 1999, Pennycook & Towill 2000). During the treatment of LS following preculture with 0.3 M sucrose, the meristematic cells are osmotically dehydrated and plasmolysed. The cells are then successively dehydrated with PVS2, producing concentrated spherical protoplasts. It is currently unknown what the actual protective mechanism is, however, the creation of these plasmolysed cells may mitigate mechanical stress caused by the severe dehydration process (Tao *et al.* 1983; Jitsuyama *et al.* 1997).

**Table 1:** Effect of sucrose concentration of preculture on recovery of shoot tips cryopreserved by vitrification

Sucrose conc	Recovery (%)			
	1day		3 days	
	+LN	-LN	+LN	-LN
0.1	0 <sup>a</sup>	21.5 <sup>c</sup>	43.3 <sup>d</sup>	65.2 <sup>f</sup>
0.3	16.7 <sup>bc</sup>	42.9 <sup>d</sup>	65.5 <sup>e</sup>	70.0 <sup>f</sup>
0.5	0 <sup>a</sup>	14.3 <sup>b</sup>	10.0 <sup>b</sup>	57.1 <sup>e</sup>
0.7	0 <sup>a</sup>	13.4 <sup>b</sup>	0 <sup>a</sup>	35.8 <sup>d</sup>

Material: Grape (cv. Fantasy seedless). Excised shoot tips were precultured with four different concentrations of sucrose for 1 or 3 days, and then exposed to a mixture of 2 M glycerol plus 0.4 M sucrose for 20 min at 25 °C. These osmoprotected shoot tips were dehydrated with PVS2 for 80 min at 0 °C before being plunged into LN. -LN: treated control without plunging into LN. Mean separation by Duncan's multiple range test, at  $p = 0.05$ .

**Table 2:** Effects of preculture and LS treatment on recovery of shoot tips cryopreserved by vitrification

Preculture	LS treatment	Recovery (%)	
		+LN	-LN
-	-	0 <sup>a</sup>	0 <sup>a</sup>
-	+	12.0 <sup>c</sup>	35.0 <sup>d</sup>
+	-	0 <sup>a</sup>	4.0 <sup>b</sup>
+	+	70.0 <sup>e</sup>	65.0 <sup>e</sup>

**Table 3:** Recovery of vitrified shoot tips cooled to -196 °C by two different procedures of vitrification

Dehydration procedure	Recovery (%)	
	+LN	-LN
1-step	65.0 <sup>a</sup>	68.5 <sup>a</sup>
2-step	80.5 <sup>b</sup>	75.0 <sup>b</sup>

Material: Grape (cv. Fantasy seedless). Shoot tips were precultured with 0.3 M sucrose for 3 days at 25 °C, and then exposed to a mixture of 2 M glycerol plus 0.4 M sucrose for 20 min at 25 °C. They were dehydration with PVS2 for 80 min at 0 °C (1- step), or a 50% PVS2 (half strength of the solution) for 30 min at 0 °C followed by full strength PVS2 for 50 min at 0 °C (2- step) before being plunged into LN. -LN: treated control without plunging into LN. Mean separation by Duncan's multiple range test, at p = 0.05.

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# Online Mode of Education and Constraint in Diploma Agriculture College, ICAR - Krishi Vigyan Kendra Bidar, Karnataka

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## ABSTRACT

The present study was conducted at Diploma Agricultural college, ICAR - Krishi Vigyan Kendra, Bidar district of Karnataka state during 2022 to analyze the online mode of education of diploma I<sup>st</sup> year and II<sup>nd</sup> year students (2021 batch) of Bidar district. During Covid-19 pandemic lockdown situation to continue the process of teaching and learning, the students and teachers got connected through online platforms, virtual mode of education itself being a new concept to learn within a short period of time virtual mode has been proven promising alternate way although there are many drawbacks compared to physical mode of education. In this study it was found most of teacher conducted online class through Google meet platform. Nearly half (48.57 %) of the students had an availability of internet connection as a major constraint and more than half (54.50 %) of the respondents responded that the virtual mode of education was good whereas (38.00 %) found it moderate and (7.50 %) students found virtual mode poor.

## HIGHLIGHTS

- ① The pandemic has utterly disrupted an education system. Approximately 264 million children and adolescents are not in school.
- ② The study was conducted during 2021-22 in Diploma Agriculture college Bidar, ICAR-Krishi Vigyan Kendra Bidar district of Karnataka state.
- ③ Total number of student during 2022 was 70, from 1<sup>st</sup> year 49 Students and 21 students from 2<sup>nd</sup> year were taken.
- ④ The online classes were conducted regularly at Agriculture Diploma College, Bidar for all courses during covid-19 pandemic.

**Keywords:** Google meet, Online mode, Pandemic, Internet, Virtual mode, Smart gadgets

It is clear that this pandemic has utterly disrupted an education system. Approximately 264 million children and adolescents are not in school (UNESCO, 2017), and this pandemic made this situation further worst. As the COVID-19 pandemic spreads, there has been an increasing move towards teaching online because of shutting down of schools, colleges and universities for an indefinite time as the only option left (Kaur 2021). Therefore, this is the time to gravely rethink, revamp and redesign our education system in much demanding need

of unprecedented current situation. Informal and non-formal education is also tremendously affected. However, it is a well-established assumption that no pedagogical approach can replace the peak position of formal education due to having teacher-taught

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direct interaction. But, the aftermath of COVID-19 crisis, online education became a pedagogical shift from traditional method to the modern approach of teaching-learning from classroom to Zoom, from personal to virtual and from seminars to webinars. Previously, e-learning, distance education and correspondence courses were popularly considered as the part of non-formal education, but as of now, it seems that it would gradually replace the formal education system if the circumstances enduringly persist over the time (Tam and El-Azar 2020).

After four months of online experiences, a paradigm shift has occurred with online teaching, gaining prominence to have near permanence even after COVID-19 pandemic leading to refreezing. Refreezing step is inevitable for integrating technology in our teaching-learning process that enables us to teach students with the methods in which they would not only feel comfortable but also, they can match the demands of technology in 21<sup>st</sup> century (Mishra, Gupta and Shree 2020).

## METHODOLOGY

The study was conducted during 2021-22 in Diploma Agriculture college Bidar, ICAR - Krishi

Vigyan Kendra Bidar district of Karnataka state. Total number of student during 2022 was 70, from 1<sup>st</sup> year 49 Students and 21 students from 2<sup>nd</sup> year were taken. The online classes were conducted regularly at Agriculture Diploma College, Bidar for all courses during Covid-19 pandemic. The reporting was on weekly basis and submitted in specific format with information on total number of teachers conducted online classes, total number of sessions, total number of students attended with percentage of attendance along with mode opted indicating online platforms used. For easy study made periods of starting one weak and later month wise.

## RESULTS AND DISCUSSION

From the table 1, Majority (95.24%) of student attended class during last period of time of lockdown because at the end student come to know how to use google meet. And most of them used google meet as Mode opted for teaching because the google meet accessed through gmail and institution ID. The google meet accessed through college mail has lot of applications as there was no restriction with respect to time and any number of sessions

**Table 1:** Progress of Delivery of Classes through Online

Sl. No.	Period	Class	Total number of teacher conducted classes	Total no. of students attended	Total Strength of the class	Percentage of students attended classes (%)	Mode opted
1	24-04-2021 to 30-04-2021	I year Diploma (Agri.)	7	37	49	75.51	Google meet
		II year Diploma (Agri.)	6	16	21	76.19	Google meet
2	1-05-2021 to 31-05-2021	I year Diploma (Agri.)	9	39	49	79.59	Google meet
		II year Diploma (Agri.)	8	18	21	85.71	Google meet
3	1-06-2021 to 30-06-2021	I year Diploma (Agri.)	8	40	49	81.63	Google meet
		II year Diploma (Agri.)	8	19	21	90.47	Google meet
4	1-07-2021 to 31-07-2021	I year Diploma (Agri.)	9	44	49	89.79	Google meet
		II year Diploma (Agri.)	7	19	21	90.47	Google meet
5	1-08-2021 to 9-08-2021	I year Diploma (Agri.)	8	44	49	89.79	Google meet
		II year Diploma (Agri.)	8	20	21	95.24	Google meet



**Table 2:** Progress in online classes from different Departments

Sl. No.	Subject/Department	Courses	Total number of teacher conducted classes	Total no. of students attended	Total Strength of the class	Percentage of students attended classes (%)	Mode opted
1	Agronomy	DAGR-101 (1+1)	1	45	49	91.84	Google meet
		DAGR-202 (1+1)	1	19	21	90.48	Google meet
2	Agri. Entomology	DAET-101 (1+1)	1	44	49	89.79	Google meet
		DAET-201 (1+1)	1	18	21	85.71	Google meet
3	Soil Science & Agricultural Chemistry	DSAC-101 (1+1)	1	45	49	91.84	Google meet
		DSAC-201 (1+1)	1	19	21	90.47	Google meet
4	Agricultural Economics	DAEC-102 (1+1)	1	45	49	91.83	Google meet
5	Agricultural Engineering	DAEG-102 (1+1)	1	48	49	97.95	Google meet
6	Plant Pathology	DPAT-101 (1+1)	1	43	49	87.75	Google meet
7	Seed science and Technology	DSST-101 (1+0)	1	46	49	93.87	Google meet
8	Animal science	DASC-101 (1+1)	1	47	49	95.91	Google meet
9	Fisheries	DFSH-101 (0+1)	1	49	49	100	Google meet
10	Vermicompost production	DAET-202 (0+1)	1	41	49	83.67	Google meet
11	Agricultural Extension	DAEX-101 (1+1)	1	44	49	89.79	Google meet

**Table 3:** Constraints faced by the Students during online Class (n=70)

Sl. No.	Constraints	f	%	Ranks
1	Availability of constant internet connection	34	48.57	I <sup>st</sup>
2	Availability of cellular network	30	42.86	II <sup>nd</sup>
3	Electricity problem	12	17.14	IV <sup>th</sup>
4	Availability of basic study material (Viz., study room, table, chair)	25	35.71	III <sup>rd</sup>
5	Availability of smart gadgets to attend online classes	10	14.29	V <sup>th</sup>

*f* = frequency, % = per cent.

can be conducted. From table 2, majority of 91.84% of student attended Agronomy and Soil science department Classes. These finding are in line with the research of Jayateertha, Diwan *et al.* (2021).

### Constraints faced by the respondents

From the table 3, it revealed that, nearly half (48.57%) of students were indicated Availability of Constant Internet connection as a constraints followed by Availability of network (42.87%), 35.71% of student indicated Availability of Basic study material (viz., room, table, chair) as a problem, Electricity problem (17.14%) and Availability of smart gadgets (14.29%)

as a constraint faced by student during online class this is due to as many students were staying in rural areas and need to move to the area wherever better mobile signal will be there. It was possible only for theory classes as no practical class could be conducted.

**Table 4:** Student's responses to online mode of education (n=70)

Parameter	Percentage (%)
Good	54.5
Moderate	38
Poor	7.5

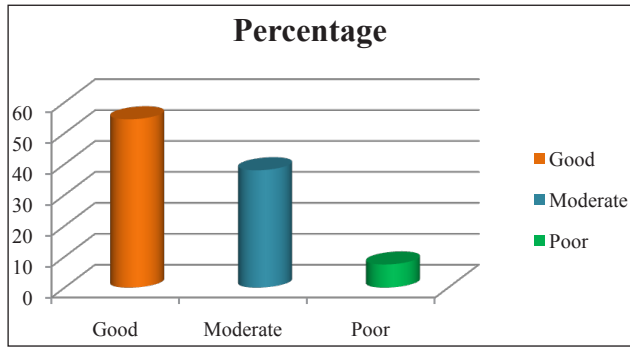


Fig. 1: Graph representing the Students responses to online mode of education

**Student feedbacks were also collected using the following parameters**

Sl. No	Parameters
1	Student. No.
2	Name of the course
3	Course number and credit hours
4	Name of the Teacher
5	Name of the application used(more preferred)
6	Mode of presentation (PPT/white board/notes share)
7	Network connectivity (Good/ moderate/ poor)
8	Voice clarity (Good/ moderate/ poor)
9	Interaction (yes/No)
10	Satisfactory level with online mode of teaching inthe present Covid -19 situation(Good/ moderate/poor)
11	Any other remarks by student

**CONCLUSION**

Covid-19 pandemic disturbed every field’s regular activities among which education is one of the major field, education has its own academic time limits which suddenly got disturbed by the hit of pandemic resulting in lockdown situation. To tackle this never experienced hard times online / virtual mode of education method came up as an alternate way to continue the educational activities. Virtual mode of education itself being a new concept to learn within a short period of time virtual mode has been proven promising alternate way although there are many drawbacks compared to physical mode of education for time being virtual mode of was only the best way to continue the education.

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# Morphological and Biochemical Markers for Dry Root Rot (*Macrophomina phaseolina* (Tassi) Goid) Resistance in Brinjal

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## ABSTRACT

Brinjal (*Solanum melongena* L.) is one of the important indigenous vegetable crop grown round the year in India. However, enhanced yield on cultivar seldom satisfy consumers of the region unless the preferred colour, size and shape is fulfilled. The root rot caused by *Macrophomina phaseolina* (Tassi) Goid, registered with yield loss ranged from 10 to 70.5%. It is soil borne with a wide host range (75 plant families and more than 500 species worldwide) registered the most challenging task as it prevailed from arid to temperate and from low to high rainfall regions. The over expression of antifungal genes (i.e., chitinase,  $\beta$ -1, 3-glucanase and stilbene synthase) governing enzymes had their roles in enhancing resistance for dry root rot in several crops. Ascertaining commercial varieties and wild species of *Solanum* against dry root rot disease recorded with *S. torvum* with no disease incidence (0) against the other wild species *S. incanum* (38.60%), *S. xanthocarpum* (42.80%), *S. viarum* (64.20%) and commercial F<sub>1</sub> hybrid CO<sub>2</sub> with the highest disease incidence of 78.50 % strongly proved commercial varieties are highly susceptible. The resistance nature of *S. torvum* is attributed by the highest phenols (17.05 mg g<sup>-1</sup>), Ortho dihydroxy phenol (12.95 mg g<sup>-1</sup>), Peroxidase (3.12 OD min<sup>-1</sup> g<sup>-1</sup>), Polyphenol oxidase (3.18 OD min<sup>-1</sup> g<sup>-1</sup>), Phenylalanine ammonium lyase (15.39 nmol of trans cinnamic acid min<sup>-1</sup> g<sup>-1</sup>) and Acid phosphatase (117.15  $\mu$  moles p-nitrophenol min<sup>-1</sup> mg<sup>-1</sup>). The morphological markers distinguishing the *S. torvum* against the other species were the highest plant growth (310 cm), root system (48.5cm), leaf size (27.5 × 16.8 cm) with thorns on leaf, shoot and fruit strongly indicated that breeding improved varieties with these morphological and biochemical markers would result with dry root disease resistant for sustainable brinjal cultivation.

## HIGHLIGHTS

- Brinjal is an important indigenous vegetable infested severely and caused yield loss ranging from 10-70.5 % by dry root rot.
- Five wild species of *Solanum* compared with F<sub>1</sub> hybrid CO<sub>2</sub> revealed that percent disease index of 0-64.20% in wild species and 78.50% in F<sub>1</sub> Hybrid of brinjal.
- The vigorous growth of root and leaves with enzymatic activity of *S. torvum* can be ideal morphological and biochemical marker for resistant breeding of dry root rot.

**Keywords:** Brinjal, wild *Solanum* species, dry root rot, screening, host plant resistance

Brinjal (*Solanum melongena* L.) is one of the important and popular vegetable crops grown throughout the year all over the country, except in high altitudes. It is known as the poor man's vegetable and also called as eggplant, garden egg and internationally aubergine. In India, next to potato and tomato, brinjal occupies third position among vegetables. A number of cultivars are grown throughout the country depending upon the yield, suitability for the region and

consumer's preference with respect to the colour, size and shape of the various cultivars. The unripe fruit is primarily used as cooked vegetable for the preparation

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of various recipes in different regions of the world. It is a favourite dish in South Indian curries. Since, brinjal has a low calorific value (24 kcal per 100g) and high potassium content (2 mg per 100g), it is suitable for diabetes and obese patients (Bajaj *et al.* 1981). Brinjal is a vegetable having hypoglycemic activity, which means antidiabetic action (Ramachandran 1999). Brinjal is widely consumed in tropical and subtropical regions of the globe. India, Bangladesh, Pakistan, China and Philippines are the major growers of brinjal. It is a hardy crop, easy to cultivate and gives higher yield. In India, brinjal cultivation is estimated in an area of 0.75 million ha with an annual production of 13.15 million tonnes with productivity of 17.5 tonnes ha<sup>-1</sup> (www.nhb.gov.in).

Drastic reduction in yield might be due to several biotic and abiotic factors. Among the biotic factors hampering the production of brinjal, root rot caused *Macrophomina phaseolina* are most important. It is an anamorphic and soil borne fungus with a wide host range that includes 75 plant families and more than 500 species worldwide (Haseeb and Archana 2009). Many economically important plants including legumes, cereals, vegetables, fruits and fibre crops were attacked by *M. phaseolina*, a causal agent of charcoal rot disease (Kunwar *et al.* 1986; Sinclair and Backman 1986; Smith and Carvil 1997). This remains to be a challenging task in terms of management, since it is of soil borne nature. *Macrophomina phaseolina* is distributed worldwide and is prevalent in arid, sub-tropical and tropical climates, especially in the areas with low rainfall and high temperature. Dry root rot of brinjal may incite a loss of 10 per cent in early harvested crop, which may go up to 70.5 per cent in the late harvested crop exposed to hot weather conditions (Thirumalachar 1953). Commercial cultivars of brinjal invariably registered susceptibility for root rot diseases, which demands the use of wild forms in breeding crop plants, particularly to obtain vigour and resistance (Sarvayya 1936). But so far no variety has been developed with resistance to root rot in brinjal by utilizing the wild type as one of the parents even though a number of attempts have been made to make interspecific hybrids and study their progenies.

Characters are chosen on criteria based on their ease for observation, availability and usefulness in classifying and identifying organisms. Hence,

these morphological markers allow assessment of genetic variability based on individual phenotypic difference yet there are limitations associated to these markers. Some characters are not modified by environmental factors and have a genetic basis such that they are unlikely to change readily (Deborah 1998). These may be referred to as constant characters and are highly heritable. Similarly, phyto-chemical are valuable tools for taxonomic differentiation within species or for evaluating the effect of environmental factors (Hawkes 1992) as evidenced in wheat breeding based on  $\gamma$ -gliadin content for pasta quality (Damidaux *et al.* 1978). Variation in biosynthesis of these metabolites could be a result from both genetic and environmental factors, which play important roles in the development of phenotypic variations in plants. Hence, there is an urgent need to screen the wild forms to identify the morphological and biochemical markers linking with root rot disease resistance is felt imperative.

## MATERIALS AND METHODS

The present investigation was carried out at the Field Research Unit of Department of Vegetable Crops, College Orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, which is situated at 11° N latitude and 77° E longitude and at an elevation of 426.6 m above MSL. An account of experimental methodology has been presented below.

### Materials and its evaluation method

The experimental materials consisted of five wild *Solanum* species used in this study are furnished in Table 1. The seeds of the above mentioned five wild species were sown in plastic cups and watered regularly. Among these species, all five species germinated except few. The germinated plants were transplanted to main field. Based on the morphological characters and performance in field (Table 2). The experiment was conducted in RBD with three replications.

### Preparation and maintenance of *Macrophomina phaseolina* culture

The root rot pathogen *Macrophomina phaseolina* was isolated from affected brinjal plant variety CO<sub>2</sub> collected from TNAU, Coimbatore. The stock culture was maintained in Potato Dextrose Agar

**Table 1:** Details of wild *Solanum* species used in the study

Wild species	Description	Source
<i>S. toroum</i>	<ul style="list-style-type: none"> <li>Turkey berry</li> <li>Plants are 2-3m in height and 2 cm in basal diameter</li> <li>Bushy, erect and spiny &amp; perennial</li> <li>Fruits are berries that borne in clusters of tiny green spheres.</li> </ul>	Alandurai region (foot hills of western ghats, Coimbatore.)
<i>S. viarum</i>	<ul style="list-style-type: none"> <li>Tropical soda apple</li> <li>Bushy, prickly herbaceous, perennial</li> <li>Fruits are globose berry, green with dark veining.</li> </ul>	Indian Institute of Horticultural Research, Bangalore (Karnataka)
<i>S. xanthocarpum</i>	<ul style="list-style-type: none"> <li>Yellow berried night shade</li> <li>Very spiny diffused herb</li> <li>Fruits are globular drooping, yellow or pale in colour, with green veins surrounded by enlarged calyx.</li> <li>Seeds are glabrous.</li> </ul>	Anaikatti region (Bordering Kerala state) (Western Ghats)
<i>S. incanum</i>	<ul style="list-style-type: none"> <li>Night shade plant</li> <li>Herbaceous 50 cm height.</li> <li>Leaves entire to deeply lobed.</li> <li>Prickles on the midrib and lateral vines may or may not be present.</li> <li>Fruit spherical, green often striped or mottled with white, turning yellow to orange brown when ripe.</li> </ul>	Anaikatti region (Bordering Kerala state) (Western Ghats)
<i>S. elaeagnifolium</i>	<ul style="list-style-type: none"> <li>Silver leaf bitter apple</li> <li>Multi stemmed, up to 1 m tall,</li> <li>Extensive root system spreading to over 2 m deep.</li> <li>Fruits are irregularly dehiscent berry, initially spherical, green (with white patches) and fleshy, drying and becoming yellow to orange at maturity.</li> </ul>	Palani
COBH 2	<ul style="list-style-type: none"> <li>Plants are semi-erect (100-110 cm).</li> <li>Fruits are medium sized, slightly oblong and dark violet in colour.</li> <li>Fruits borne in clusters (each 55-60 g).</li> <li>The hybrid possesses tolerance to shoot and fruit borer incidence.</li> </ul>	Dept. of Vegetable Crops, TNAU, Coimbatore.

**Table 2:** Wild *Solanum* species for morphological marker differentiation

Wild species	Plant height (cm)	No. of primary branches	Leaf length (cm)	Leaf breadth (cm)	Presence of thorns			Root length (cm)
					Leaves	Shoot	Fruit	
<i>S. erianthum</i>	106.50	3.00	43.50	20.10	-	-	-	42.50
<i>S. xanthocarpum</i>	58.00	3.00	12.50	10.55	+	+	+	30.51
<i>S. incanum</i>	65.20	2.00	10.75	9.15	+	+	+	32.10
<i>S. melongena</i> (Hilly)	89.50	2.00	27.10	19.15	-	-	-	34.00
<i>S. elaeagnifolium</i>	75.80	2.00	11.50	3.00	-	+	+	21.50
<i>S. viarum</i>	125.00	2.00	15.00	17.50	+	+	+	30.00
<i>S. toroum</i>	310	2.00	27.50	16.8	+	+	+	48.50
<i>S. sysimbrifolium</i>	Germinated but no growth under field condition							
<i>S. capsicoides</i> & <i>S. violaceum</i>	No germination							

\* Mean of five replications; Figures in parentheses are arc sine transformed values.

**Table 3:** Dry root rot incidence on wild *Solanum* species under field condition

Rootstock	Root rot incidence (%)		
	30* DAP	45* DAP	60* DAP
<i>S. toroum</i>	0	0	0
<i>S. viarum</i>	64.20 (53.26)	76.90 (61.30)	86.47 (68.49)
<i>S. xanthocarpum</i>	42.80 (40.86)	68.50 (55.87)	73.19 (58.83)
<i>S. incanum</i>	38.60 (38.41)	52.70 (46.54)	63.87 (53.06)
<i>S. elaeagnifolium</i>	50.40 (45.23)	70.30 (56.99)	84.18 (66.62)
CO	78.50 (62.41)	89.20 (70.94)	92.29 (74.13)



plates and slants, further sub cultured at monthly interval.

### Evaluation of wild forms to dry root rot pathogen (*Macrophomina phaseolina*) under artificial inoculation

Pot culture experiment was conducted under glasshouse condition at College Orchard, TNAU, Coimbatore. Wild forms along with check CO<sub>2</sub> were planted in earthenware containing five kilogramme of sterilized pot mixture (Red soil: Sand: FYM in 2:2:1 ratio). The plants involved in the study were inoculated with *Macrophomina phaseolina* @ hundred grammes per pot on fifteen days after planting. The plants were observed for wilting symptoms from 30, 45 and 60 DAP. Observations were recorded for morphological, growth and quality characters on individual plant basis. The data collected on single plant basis were subjected to statistical analysis to get information on their mean performance.

## RESULTS AND DISCUSSION

The genetic markers comprises of morphological, biochemical and molecular kind, where the former two markers can be applied at field level and less expensive. Several succesful application of morphological and biochemical markers have applied and bred superior varieities in crops like wheat (Damindaux *et al.* 1978) and onion (Cramner and Harvey 1999) for quality improvements. However, the indogenous crop brinjal had no resistant variety for dry root rot disease yet. The dry root rot pathogen *M. phaseolina* (Tassi.) Goid is an important soil borne phytopathogen causing dry root rot disease in many crop plants including brinjal. The pathogen survives well in light textured soil and under moisture stress condition (Bhatia *et al.* 1998). Further, eradication of soil borne plant pathogen is a challenge because they can survive for a longer period in the absence of the host plants through sclerotia. It caused serious economic loss ranging from 20 to 30 per cent under dry warm conditions (Sandhu and Singh 1998). The soil borne pathogen entered plant through wounded roots or natural opening (secondary roots) and invaded the vascular tissues inducing partial or complete rotting (McCreight *et al.* 1993). Under such circumstances, using of wild species for plant pathogen is an attractive preposition as it mimics

the natural way of balancing population of living microorganism and helps to increase the yield by the suppression of pathogen inoculum, protect plants to resist pathogen and also to attain a safe and clean environment. To assess the resistance of wild *Solanum* species against the pathogen *M. phaseolina*, soil inoculation method was used in screening the genotypes (Kaur *et al.* 2004). In view of this fact, the wild *Solanum* species against dry root rot pathogen were screened under pot culture and field conditions to identify the resistant species for further studies.

### Percent disease index (PDI)

The analysis of variance in the evaluation programme in a completely randomized design and randomized block design showed significant differences among the treatments for all the characters (Table 2 & 3). The percent disease index (PDI) of five wild species at different intervals of 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> days after planting under pot culture conditions. At 30 DAP, wild species showed wider range from 0 to 53.26. When the wild species were compared among themselves for dry root rot incidence, there was no incidence in *S. torvum*, very least per cent observed in *S. incanum* (38.60) and *S. xanthocarpum*. Whereas *S. viarum* recorded the highest value of 64.20 per cent for root rot incidence at 30 DAP. Overall the least performed variety CO<sub>2</sub> recorded the highest per cent incidence for root rot with 78.50 per cent. At 45 DAP, there was no incidence in *S. torvum*, followed by *S. incanum* (52.70 per cent). *S. viarum* recorded the highest per cent disease incidence (76.90) and the susceptible check CO<sub>2</sub> recorded the highest per cent (89.20 per cent) incidence. Similarly, the per cent disease incidence of root rot observed at 60 DAP was ranged from 0 to 86.47 percent (Table 9). There was no disease incidence at 60 DAP in *S. torvum* followed by *S. incanum* (63.87 per cent), whereas the highest per cent of root rot incidence was registered by *S. viarum* (86.47 per cent) as against CO<sub>2</sub> which recorded the highest root rot incidence (92.29 per cent). The differential PDI among the wild species clearly revealed that the wild species *Solanum torvum* could be considered as 'highly resistant' species for *M. phaseolina*, and there were no earlier reports in solanaceous crops regarding resistance to *M. phaseolina* and this would be the first record of evidence to show that this



species is resistant to *M. phaseolina*. However, the earlier reports revealed the resistance of *S. torvum* to other soil borne diseases *viz.*, *Verticillium* wilt, *Fusarium* wilt, bacterial wilt and *Phomopsis* blight (Yamakawa 1982). The resistance nature of *S. torvum* is attributed by the higher amount of lignified cells in the roots, more number of functional roots and production of fungal cell degrading chemicals and enzymes.

### Morphological factors

In the past several morphological markers have been used in crop improvement such as glossy foliage is caused by the absence of wax on the surface of the leaf (Molenaar 1984), flower morphology (exposed anthers) for male sterility (Jones 1944) in onion. Similarly, the wild species exhibited significant difference from seed germination to plant ad root growth (Table 4). Except, *Solanum sysimbrifolium*, *Solanum capsicoides* and *Solanum violeceum* the rest wild species recorded germination. Considering plant growth, *Solanum torvum*, *Solanum viarum* and *Solanum erianthum* had higher plant height (>100.0 cm) as compared to other species. Leaf length ranged from 10.75 to 43.50 cm where *Solanum erianthum*, *Solanum melongena* and *Solanum torvum* recorded with leaf length of >20.0 cm. Similarly for leaf breadth, wider range was observed between 9.15 to 20.10 cm where four species had leaf breadth >15.0 cm. Root length too had wider range between 21.50 to 42.50 cm with *Solanum erianthum* and *Solanum torvum* recording higher values of >40.0cm. It is interesting to note that despite of vigorous plant growth in *Solanum erianthum* and *Solanum torvum*, the former had no thorns on leaves, shoots and fruits as against the latter species which had thorns respectively. This finding clearly revealed that morphological marker too is more important to resistance to diseases despite the growth

parameters are similar. The typical growth pattern of *S. torvum* and its resistant nature against dry root rot is supported by Yan *et al.* (2011) that the possible effects of plant height on fusarium head blight (FHB) resistance in wheat assessed using near-isogenic lines (NILs) for several different reduced-height (*Rht*) genes, where tall isolines gave better type I resistance than their respective dwarf counterparts.

### Biochemical factors

The induced multiple defense responses are elicited when plants are exposed to biotic stresses such as attack by herbivores or pathogens (Sarosh and Meijer 2007). Higher plants have intricate mechanisms enabling to respond to environmental changes, most likely established over a long period of evolution as sessile organisms (Wu *et al.* 2007). Phytohormones may participate in stress perception signaling and possibly initiate a cascade of stress-induced responses (Chandler and Robertson 1994). It is obvious that the biochemical factors are more important than morphological and physiological factors in conferring non-preference and antibiosis (Prabhu *et al.* 2008). Occurrence at lower concentration or total absence of such biochemicals leads to non-preference, a form of insect resistance (Singh 1983). The biochemical constituents like glycoalkaloid (solasodine), phenols, ortho-dihydroxy phenols, phenolic oxidase enzymes *viz.*, polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, IAA oxidase and acid phosphatase are available in brinjal and these biochemical constituents possess insect resistant properties (Kalloo 1988).

The present study revealed that the total phenol content in roots was estimated after challenge inoculation of pathogen among the wild species

**Table 4:** Dry root rot incidence on wild *Solanum* species under field condition

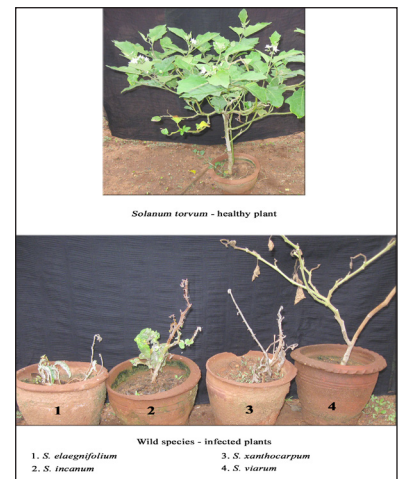
Rootstock	Root rot incidence (%)		
	30* DAP	45* DAP	60* DAP
<i>S. torvum</i>	00	0	0
<i>S. viarum</i>	32.60(34.82)	50.48(45.28)	72.98(58.69)
<i>S. xanthocarpum</i>	25.10(30.06)	40.56(39.56)	52.93(46.68)
<i>S. incanum</i>	18.29(25.32)	28.16(32.05)	43.37(41.19)
<i>S. elaeagnifolium</i>	30.42(33.47)	48.25(43.99)	68.90(56.12)
CO <sub>2</sub>	40.50(39.52)	62.34(52.15)	83.74(66.28)

**Table 5:** Enzymes content in wild *Solanum* species roots under field condition

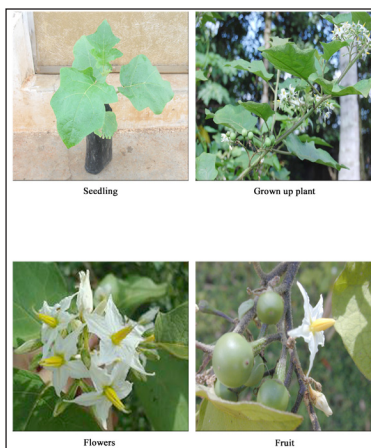
Wild species	96 Hours after inoculation					
	Phenols (mg g <sup>-1</sup> )	Ortho dihydroxy phenol (mg g <sup>-1</sup> )	Peroxidase (changes in OD min <sup>-1</sup> g <sup>-1</sup> of roots)	Polyphenol oxidase (changes in OD min <sup>-1</sup> g <sup>-1</sup> of roots)	Phenylalanine ammonium lyase (nmol of trans cinnmic acid min <sup>-1</sup> g <sup>-1</sup> of roots)	Acid phosphatase (mmoles p-nitrophenol min <sup>-1</sup> mg <sup>-1</sup> protein of roots)
<i>S. torvum</i>	19.84	15.84	3.65	3.58	16.10	131.88
<i>S. viarum</i>	11.54	8.05	1.56	1.85	9.98	99.07
<i>S. xanthocarpum</i>	16.97	10.51	2.07	2.32	13.14	126.06
<i>S. incanum</i>	17.84	12.46	2.25	2.38	13.40	127.31
<i>S. elaeagnifolium</i>	12.98	8.34	1.76	2.21	11.35	110.01
CO <sub>2</sub>	10.98	5.21	0.89	0.914	5.68	67.13



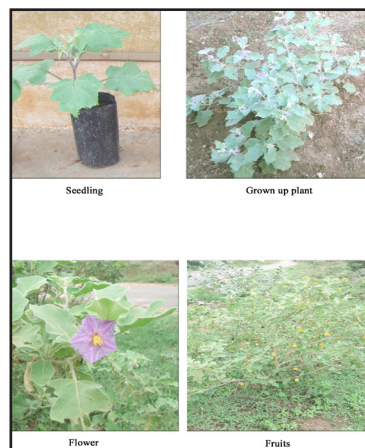
Wild species of Solanum



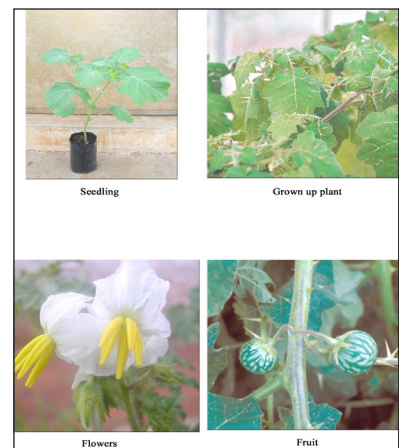
Screening of wild species for dry root rot disease



*S. torvum*



*S. incanum*



*S. viarum*

**Fig. 1:** Wild species of *Solanum* and its screening for dry root rot disease resistance

(Table 5). The highest phenol was recorded in *S. torvum* (37.36 mg g<sup>-1</sup> of root) followed by *S. incanum* (23.40 mg g<sup>-1</sup> of root) against *S. viarum* (13.14 mg

g<sup>-1</sup> root) and CO<sub>2</sub> (11.56 mg per g of root). The present finding strongly supported by Tepper and Anderson (1984) who found that phenolics in higher





concentration were toxic to plant cell themselves and higher content of total phenol observed might be responsible for checking symptom development after inoculation with pathogen. The increased production of phenolics which were cytotoxic might have contributed to increased cell death in susceptible genotypes and thereby its hypersensitive reaction to the diseases. Similarly, the ortho-dihydroxy phenol content was found highest in *S. torvum* (17.63 mg g<sup>-1</sup>) followed by *S. incanum* (15.19 mg g<sup>-1</sup>) against *S. viarum* (10.23 mg g<sup>-1</sup>) and CO<sub>2</sub> (4.57 mg g<sup>-1</sup> of root). A similar differential level of OD ortho-dihydroxy phenols were observed in the resistant and moderately resistant genotypes as compared to susceptible ones in chillies (Prasath and Ponnuswami 2008) against *Colletotrichum capsici*.

The peroxidase activity was found highest in wild species *S. torvum* (3.22 changes in OD min<sup>-1</sup>g<sup>-1</sup>) followed by *S. incanum* (2.44 changes in OD min<sup>-1</sup>g<sup>-1</sup>) against the wild species *S. viarum* (1.42 changes in OD min<sup>-1</sup>g<sup>-1</sup>) and CO<sub>2</sub> (0.93 changes in OD min<sup>-1</sup>g<sup>-1</sup> in root). Peroxidases have been implicated in a number of physiological functions that may contribute to resistance including exudation of hydroxy cinnamyl alcohol into free radical intermediates (Gross 1980) and lignification (Walter 1992) and also associated with deposition of phenolic compounds into plant cell walls during resistant interactions (Graham and Graham 1991). Similarly, polyphenol oxidase activity was found highest in wild species *S. torvum* (3.17 changes in OD min<sup>-1</sup>g<sup>-1</sup>) followed by *S. incanum* (2.55 changes in OD min<sup>-1</sup>g<sup>-1</sup>) against *S. viarum* (1.26 changes in OD min<sup>-1</sup>g<sup>-1</sup>) and CO<sub>2</sub> (1.14 changes in OD min<sup>-1</sup>g<sup>-1</sup> in root). The above finding is proved by enhancement of PPO activity in wild species which catalyses the last step of biosynthesis of lignin and other oxidative phenols (Goodman *et al.* 1986; Saharan *et al.* 2001; Joshi *et al.* 2004) in cluster bean against charcoal rot. The activity of phenylalanine ammonia lyase was found highest in *S. torvum* (31.79 nmol of trans cinnamic acid min<sup>-1</sup>g<sup>-1</sup>) followed by *S. incanum* (18.81 nmol of trans cinnamic acid min<sup>-1</sup>g<sup>-1</sup>) as compared to *S. viarum* (12.17 nmol of trans cinnamic acid min<sup>-1</sup>g<sup>-1</sup>) and CO<sub>2</sub> (10.71 nmol of trans cinnamic acid min<sup>-1</sup>g<sup>-1</sup>). PAL plays an important role in the biosynthesis of various defense chemicals in phenyl propanoid metabolism (Daayf *et al.* 1997). Meyer *et al.* (1999) reported that rhizosphere colonization

of *P. aeruginosa* 7 NSK 2 activated PAL in bean roots and increased the salicylic acid levels in leaves. PAL activity could be induced in plant-pathogen interactions and fungal elicitor treatment (Ramanathan *et al.* 2001).

## CONCLUSION

Several morphological and biochemical markers have been used in locating the geneotypes for yield, quality and stress tolerance in crop plants. An attempt made in brinjal for dry root rot disease resistance showed *S. torvum* with no disease incidence (0) against the other wild species *S. incanum* (38.60%), *S. xanthocarpum* (42.80%), *S. viarum* (64.20%) and commercial F<sub>1</sub> hybrid CO<sub>2</sub> with the highest disease incidence of 78.50 % strongly proved commercial varieties are highly susceptible. The resistance nature of *S. torvum* is attributed by the highest phenols (17.05mg g<sup>-1</sup>), Ortho dihydroxy phenol (12.95 mg g<sup>-1</sup>), Peroxidase (3.12 OD min<sup>-1</sup> g<sup>-1</sup>), Polyphenol oxidase (3.18 OD min<sup>-1</sup> g<sup>-1</sup>), Phenylalanine ammonium lyase (15.39 nmol of trans cinnamic acid min<sup>-1</sup>g<sup>-1</sup>) and Acid phosphatase (117.15 *m* moles p-nitrophenol min<sup>-1</sup> mg<sup>-1</sup>). Similarly, the morphological markers distinguishing the *S. torvum* against the other species were the highest plant growth (310 cm), root system (48.5 cm), leaf size (27.5 × 16.8 cm) with thorns on leaf, shoot and fruit strongly indicated that breeding improved varieties with these morphological and biochemical markers would result with dry root disease resistance.

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# Impact of Climate Change on Water Requirement and Yield of Tomato Over different Agro-climatic Zones of Tamil Nadu

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## ABSTRACT

The study was aimed to assess the impact of Climate change on water requirement and yield of tomato over different agro climate zones of Tamil Nadu. Tomato is the world's largest vegetable crop which is considered an important commercial and dietary vegetable crop. In Tamil Nadu, the tomato has greater economic importance among the vegetables since it is one of the leading commodities in agricultural exports. Successful production of Tomato in the tropics is constrained by environmental variations, especially under open field conditions. Efficient cropping districts were identified in different agro-climatic zones of Tamil Nadu based on the maximum area covered under tomato cultivation from Season and Crop Report, Department of Economics and Statistics, Government of Tamil Nadu and considered for the current investigation. Rainfall and Temperature data (1990 to 2019) was obtained from IMD and employed in the AquaCrop model for simulating the crop water requirement and yield of tomato. Results showed the spatial and temporal variations in tomato production across different agro-climatic zones of Tamil Nadu. Water use efficiency was higher (65.55%) in the High rainfall zone and lower (50.96%) in the Cauvery Delta zone. The northwestern zone was found to have more water requirement (580 mm) while the lowest water requirement of 447 mm was observed in the northeastern zone. The western zone produced the highest fruit yield (33.9 tones/ha) and the Cauvery delta zone exhibited the lowest yield (29.2 tones/ha). The elevated temperature harmed tomato yield and the water requirement of s tomato was increased due to an increase in temperature.

## HIGHLIGHTS

- Results showed the spatial and temporal variations in tomato production across different agro-climatic zones of Tamil Nadu.
- Water use efficiency was higher (65.55%) in the High rainfall zone and lower (50.96%) in the Cauvery Delta zone.
- The northwestern zone was found to have more water requirement (580 mm) while the lowest water requirement of 447 mm was observed in the northeastern zone.
- The western zone produced the highest fruit yield (33.9 tones/ha) and the Cauvery delta zone exhibited the lowest yield (29.2 tones/ha).

**Keywords:** AquaCrop, tomato yield, Water use efficiency (WUE), Water requirement (WR)

Tomato is grown as a commercial crop across the globe and it is one of the most important "protective foods" because of its special nutritive value and wide usage in Indian culinary tradition (Deuter *et al.* 2012). The estimated area and production of tomato for India are about 3,50,000 hectares and 53,00,000 tons respectively (FAO 2016). According to 'Agricultural Situation in India' Tamil Nadu

occupies the seventh position as regards the crop area (21,055 hectares) and eighth position in the production of tomato (2,32,430 tons) during 2015-

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16 (Nangare *et al.* 2015). Varied climatic conditions have a direct impact on the growth and yield of tomato with the different magnitude regionally and any climate changes also largely alter the crop water requirement between the regions (Cramer *et al.* 2018). The present investigation was carried out to assess the impact of varied climatic conditions on the productivity and water requirement of tomato in different agro-climatic zones of Tamil Nadu.

## MATERIALS AND METHODS

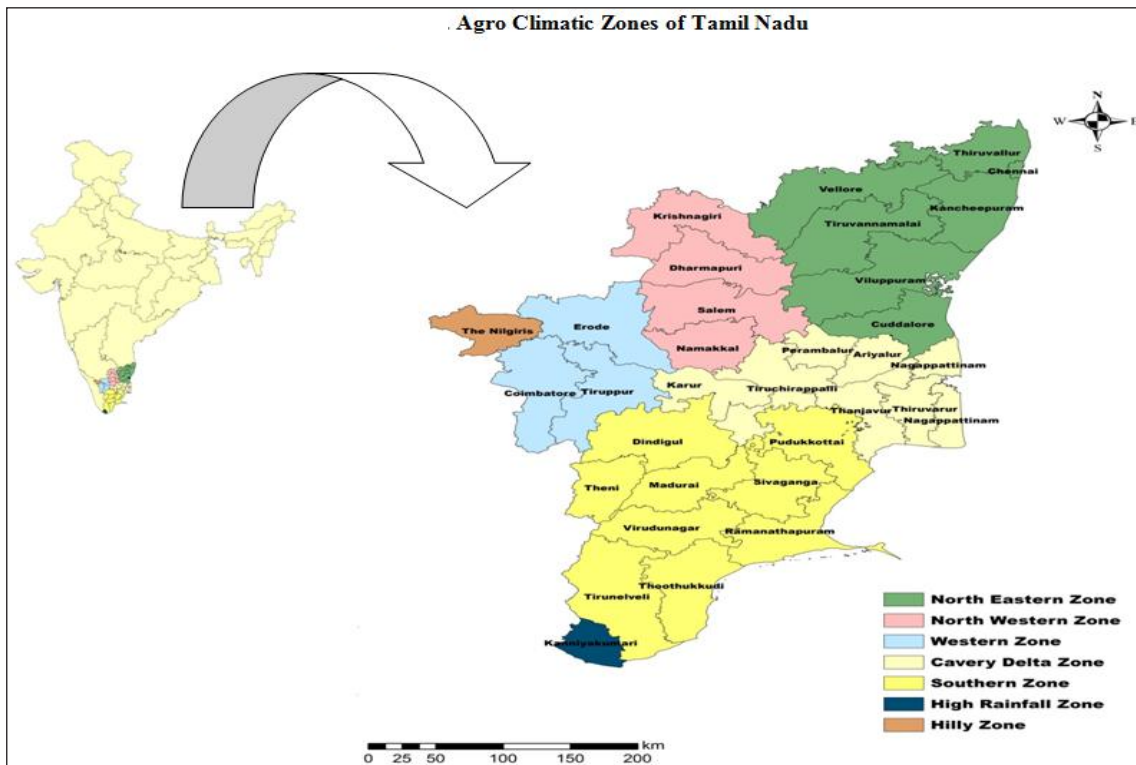
### Input requirement for setting up of AquaCrop model

AquaCrop model uses a relatively small number of explicit parameters and largely intuitive input variables, either widely used or requiring simple methods for their determination. Input consists of weather data, crop and soil characteristics, and management practices that define the environment in which the crop will be developed (Vanuytrecht *et al.* 2014). The field experiment was conducted in a Farmer’s field near Ponnaniyar dam, Mugavanur village, Vaiyampatti block of Thiruchirapalli district

(Fig. 1). The experimental site is situated at 10.51° N latitude, 78.21° E longitude and an altitude of 78.17 m above mean sea level. Based on the results from the Field experiment the AquaCrop model was Calibrated and Validated and used for further analysis.

### Assessing the impact of climate change on water requirement, WUE and yield of Tomato in different agro-climatic zones of Tamil Nadu

Efficient cropping districts were identified in different agro-climatic zones (ACZ) of Tamil Nadu based on maximum area covered under tomato cultivation from Season and Crop Report, Department of Economics and Statistics, Government of Tamil Nadu for the past ten years were taken and considered for the current investigation. Rainfall and Temperature data (1990 to 2019) was obtained from IMD and employed in the AquaCrop model for simulating the crop water requirement and yield of tomato over the past 30 years (Linker *et al.* 2016). The model simulations were made with the 2, 3 and 4 °C in temperature and analyzed for exploring the influence of elevated temperature on Tomato.



**Note:** NEZ – Northeastern zone, NWZ - Northwestern zone, CDZ – Cauvery delta zone, SZ – Southern zone, HRZ – High rainfall zone, WZ – Western zone

**Fig. 1:** Agro Climatic Zones of Tamil Nadu

### 3. RESULTS AND DISCUSSION

#### Impact of varied climate on average tomato yield over different agro-climatic zones of Tamil Nadu

Tomato production showed spatial variations among the different agro-climatic zones and inter-annual variability due to the varied climatic conditions (Table 1 and Fig. 1). The western zone (WZ) showed high yielding potential (33.9 tones/ha) with wider annual variability and the northwestern zone stood next in tomato productivity (31.8 tonnes/ha). Tomato yield was about 30 tones/ha in northeastern and high rainfall zones. Relatively low yield was noticed in southern (29.8 tones/ha) and Cauvery delta zone (CDZ) (29.2 tones/ha).

**Table 1:** Average yield (tones/ha) of tomato at different Agro-climatic zones of Tamil Nadu over 30 years (1990-2019)

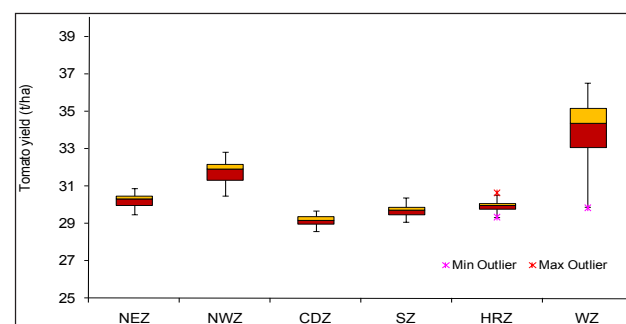
Year	Northeastern zone	Northwestern zone	Cauvery delta zone	Southern zone	High rainfall zone	Western zone
1990	30.4	31.9	29.0	29.7	29.8	35.1
1991	30.5	32.1	29.5	29.5	30.1	36.5
1992	29.9	30.5	28.7	30.0	29.8	34.1
1993	30.4	30.7	29.0	29.4	29.6	33.9
1994	30.9	32.8	29.7	30.1	30.3	35.9
1995	30.5	32.5	29.6	29.9	30.0	33.2
1996	30.2	32.0	29.2	30.1	30.3	34.4
1997	29.8	31.3	28.6	29.5	29.6	33.5
1998	30.6	31.1	29.2	29.5	29.9	29.8
1999	30.7	32.4	29.4	30.1	30.2	34.8
2000	30.5	32.2	29.4	29.1	29.3	32.0
2001	30.4	32.3	29.2	29.6	29.4	31.0
2002	30.4	32.1	29.5	29.9	30.1	35.4
2003	30.8	32.7	29.6	30.2	30.0	34.8
2004	30.0	32.2	29.2	29.8	30.1	35.5
2005	30.4	32.2	29.5	29.8	29.9	30.5
2006	30.7	32.6	29.3	30.4	30.6	35.7
2007	30.1	31.5	28.7	29.5	30.0	34.5
2008	30.0	32.1	29.1	29.9	30.1	33.0
2009	29.9	31.9	29.3	29.7	29.8	35.2
2010	30.7	32.3	29.2	30.2	30.4	32.3
2011	30.0	31.8	29.1	29.5	30.2	33.7

2012	29.5	30.9	28.7	29.8	30.1	31.4
2013	30.0	31.3	28.9	29.7	29.7	34.3
2014	30.3	31.9	29.1	29.9	30.1	35.6
2015	30.1	31.7	29.1	29.7	29.8	35.2
2016	30.3	31.7	29.3	29.8	30.2	34.4
2017	30.3	31.0	28.9	29.6	30.1	34.3
2018	30.5	32.0	29.0	29.3	29.5	34.6
2019	30.0	31.3	28.6	29.5	29.8	31.0
<b>Average yield</b>	<b>30.3</b>	<b>31.8</b>	<b>29.2</b>	<b>29.8</b>	<b>30.0</b>	<b>33.9</b>

Box and whisker plot indicates the inter-annual variation in tomato yield over 30 years (1990-2019) in Tamil Nadu.

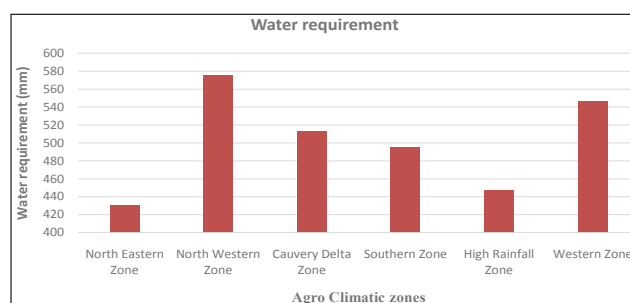
#### Water requirement under varied climate in different agro climatic zones of Tamil Nadu

Water requirement (WR) of agro climatic region was analyzed for the base period from 1990 to 2019. At agro climatic zone scale, WR varied between 447 to 580 mm. The WR was relatively higher in North western zone (NWZ) than other zones (Fig. 2).



**Fig. 2:** Influence of climate variability on tomato yield (tones/ha) at different Agro climatic zones over Tamil Nadu

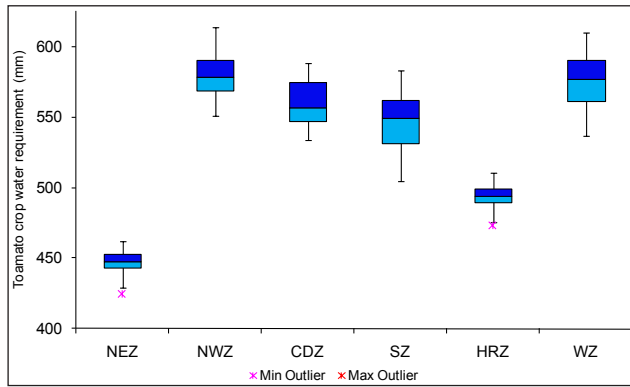
Even though water requirement was high in NWZ, more fluctuation was existed due to climate variability in the Southern zone (SZ) and WZ. Similarly, there was a higher variation in WR over the period in CDZ (Fig. 3).



**Fig. 3:** Water requirement of tomato in different agro climatic zones

**Influence of climatic variations on water use efficiency of tomato in the different agro-climatic zone of Tamil Nadu**

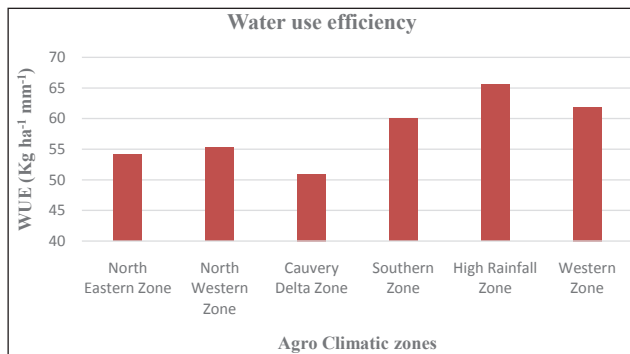
Analysis of water use efficiency (WUE) at different Agro-climatic regions over 30 years (1990 to 2019) showed that a High rainfall zone (HRZ) could use the water effectively for producing fruits (65.5 Kg ha<sup>-1</sup> mm<sup>-1</sup>) than the other zones (Fig. 4). CDZ registered less WUE (50.96 Kg ha<sup>-1</sup> mm<sup>-1</sup>).



**Fig. 4:** Variation in water requirement of as influence by climate variability

**Elevated temperature effects tomato yield, water requirement and water use efficiency of tomato in the different agro-climatic zone of Tamil Nadu**

Results clearly indicate that tomato is more sensitive to temperature (Fig. 5). Reductions in tomato yield as a result of elevated temperature are predicted to be more for SZ as well as WZ and less in CDZ (Steven Adams *et al.* 2001).

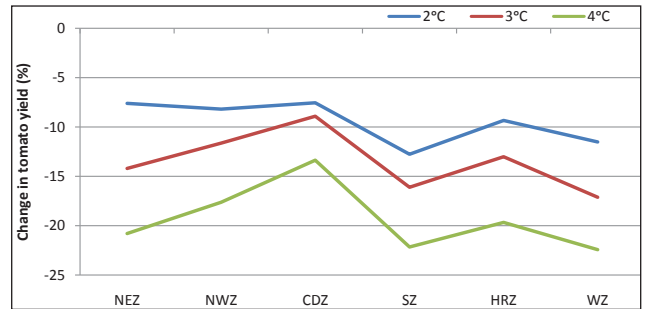


**Fig. 5:** Water use efficiency of tomato in different agro-climatic zones

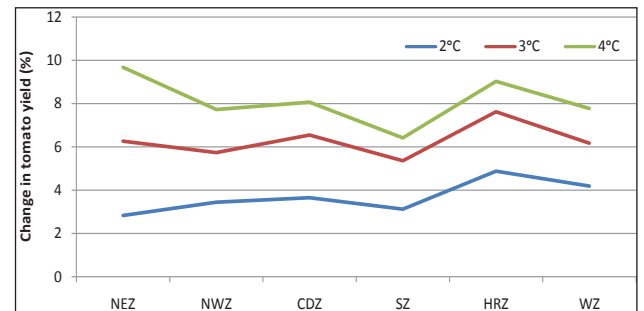
The temperature increase of 2 °C reduced the yield on an average by 9 % with a minimum of 8 and a maximum of 13% across the ACZ (Peet *et al.* 1997;

Srinivasa Rao 1995; Kallou *et al.* 2001; Sato and Thomas, 2002; Erickson and Markhart 2002; Sato 2006; Timlin *et al.* 2006; Wag- staffe and Battey 2006; Hazra *et al.* 2007; Raj Narayan 2009; Tesfaendrias *et al.* 2010; Johkan *et al.* 2011). The yield loss varied from 9 to 17 % with the mean of 13% for 3 °C increase in temperature while yield dipped in the range of 13 to 22 % with 19 % mean over the ACZ under 4 °C elevated temperature (Fig. 5).

Overall, the elevated temperature increased the WR in all the ACZ at varying magnitude. An increase of 2 °C tends to lift the WR an average of 4%. The elevated temperature by 3 °C resulted in a 6% increase in WR and the 4 °C temperature rise increased the WR by 9% (Fig. 6).



**Fig. 6:** Effect of elevated temperature on Tomato yield



**Fig. 7:** Changes in water requirement under elevated temperature

**CONCLUSION**

Tomato yield varied across different agro-climatic zones of Tamil Nadu with the maximum yield of 33.9 t/ha in the Western zone and a minimum of 29.2 t/ha Cauvery delta zone. Higher WR (580 mm) was noticed for the Northwestern zone and less WR (447 mm) for the northeastern zone compared to other agro-climatic zones of Tamil Nadu. Elevated temperature substantially reduced the tomato yield and yield is predicted to decrease by 9, 13 and 19% under 2 °C, 3 °C and 4 °C elevated temperature. The



water requirement of tomato increased by 9 % for the 4 °C rise in temperature.

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# Analysis of Resource Use Efficiency and Constraints of Gram Production in Gadchiroli District

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## ABSTRACT

The present study entitled "Analysis of Resource Use Efficiency of Gram Production in Gadchiroli District" was undertaken in three tehsils. Three villages from each tehsil were selected. From each village 10 farmers i.e. 90 farmers were selected randomly. The primary data were collected by personal interview method in pre-designed schedule regarding different resources, essential fertilizers and constraints faced by the farmers in production of gram. The results revealed that at overall level, the regression coefficient of human labour and machine charges, seeds and nitrogen and phosphorous was significant at one, five and ten per cent level, respectively. Which indicated the major contribution of these variables in output. The regression coefficient of plant protection, potash and bullock labour for gram production were found non-significant which indicated less influence of such variables on the output. Marginal value of product to factor cost ratio of all the variable was less than one. This indicates excess use of these inputs. Hence, increasing these inputs are not significant in the gram production. In the constraints faced by farmers in production of gram, destruction due to wild animals (87.78 %) ranked 1<sup>st</sup> and Infestation of Insects and pests (84.44 %) ranked 2<sup>nd</sup>.

## HIGHLIGHTS

- ① Human labour, machine charges, seeds, nitrogen and phosphorous play significant role.
- ② MVP of all the variables is less than unity, indicating excess use of these inputs cannot increase the production.

**Keywords:** Regression coefficient, Marginal value product, Resource use efficiency, Constraints

Pulses are the important crops grown in India. The different pulses play an important role in sustainable production system and household nutritional security. Pulses are edible dry seeds of plants belonging to the *Leguminosae* family. They are consumed in the form of whole seed, split grain, dehulled split grain and flour. Many different types of pulses are grown over the world. Of these, the major ones in terms of global production and consumption quantities, are the common bean, chickpea, dry pea, lentil, cowpea, mung bean, urd bean and pigeon pea. In addition, there are a large number of minor pulses that are grown and consumed in different parts of the world. While

pulses are primarily grown for human consumption, there is, in addition, substantial demand for them as animal feed in some of the developed countries. Of the various pulses, dry pea, faba bean, and lupins are widely used as animal feed. Gram is a very rich source of easily digested protein and is also rich in minerals such as magnesium, zinc, calcium, phosphorous and iron. Gram is a protein-rich supplement to cereal-based diets, especially to

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the poor in developing countries, where people are vegetarians or cannot afford animal protein. Bengal gram has a very important role in human diet in our country.

India is the largest producer of chickpea in the world with a 67 per cent share in global production. Top 10 producer countries of chickpea are India, Turkey, Russian Federation, Myanmar, Pakistan, Ethiopia, USA, Australia, Canada, Mexico. Globally pulses are grown on area of 5.9 million hectares with an annual production of 4.5 million tonnes (MT) and productivity of 759.3 kg/hectare. At world level chickpea is grown on area of 13.7 million hectares with a production of 14.2 million tonnes (MT) and productivity of 1038.4 kg/hectare (Anonymous, 2019). In India gram is grown on area of 9.44 million hectares with production of 10.13 million tonnes (MT) and productivity of 1073 Kg/hectare (Source: Ministry of Agriculture and Farmers welfare, DES, GOI). Madhya Pradesh, Rajasthan, Uttar Pradesh, Haryana, Maharashtra and Punjab are major gram producing states. Area under gram in Maharashtra is 20.43 lakh ha with production 22.40 lakh tonnes and productivity 1096 kg/ha in year 2019-20 (Source: Economic Survey of Maharashtra 2020-21). In Gadchiroli district area under gram is 13,539 hectares, with production of 10,778 tonnes and the productivity is 796.03 kg/ha in the year 2019-20 (Anonymous 2020).

In today's world problems, both economic and social, resource and the resource efficiency play a vital role. The investors have been concerned with increasing productivity of resources by introduction of new ones that lie higher technologies. By replacing the existing production function by new ones that lie higher in output, plane, it is hoped that, this investment leads to production of more output from the same quantity of inputs, or to the same outputs from few inputs (there by releasing resource for other economic activities). This study implied that the output of average farmers could be increased by adopting the allocation of resources followed by the best practiced farmers (Rede *et al.* 2013). Many hurdles are faced for successful adoption of improved cultivation practices of gram. Gram crop is suffering from various insect, pest, disease, weed and nutrient deficiency among them the pest attack creates more losses throughout their production and farmers uses various pesticide for

production of gram. Keeping this fact in view, it is necessary to study constraints in adoption of improved cultivation practices of gram (Parmer *et al.* 2019).

## OBJECTIVES

1. To estimate the resource use efficiency in gram cultivation.
2. To identify the constraints faced by the farmers in gram production.

## MATERIALS AND METHODS

The present study was undertaken in Gadchiroli district of Vidarbha region. The district was selected purposively. Out of twelve tehsils of Gadchiroli district three tehsils were selected on the basis of highest area of gram concentrated in last five years. Tehsil-wise last fiveyears data were collected from the selected tehsils and it was found that Chamorshi, Armori and Kurkheda occupied the highest area under gram. Hence, these three tehsils were selected. Three villages were selected from each tehsil. Overall 9 villages were selected. From each village 10 farmers were selected randomly. Overall 90 farmers were selected for the present study. Selected farmers were categorized into small, medium and large farmers according to their land holding size. The Schedules was pre-tested and designed for data collection by keeping in view the objectives of the study. The data were collected for the year 2020-21 through personal interview method from selected cultivators. The collected data pertaining to the cropping pattern, input utilization, cost of cultivation and returns etc. were collected from the selected farmers.

### Analyzing procedure

The collected data were tabulated, interpreted with the help of average, percentage and summarized with the aid of statistical tools to obtain the results.

### Estimation of Resource use efficiency

The Cobb-Douglas production function was used for estimating the resources used in gram cultivation.

$$Y = ax_1b_1 \cdot x_2b_2 \cdot x_3b_3 \cdot x_4b_4 \cdot x_5b_5 \cdot x_6b_6 \cdot x_7b_7 \cdot x_8b_8$$

Where,

Y = Yield in ₹ per hectare

$a$  = Intercept

$b_1, b_2, b_3, b_4, b_5, b_6, b_7, b_8$  = Regression coefficient of respective factor as follows.

$X_1$  = Human labour in ₹/ha

$X_2$  = Bullock pair in ₹/ha

$X_3$  = Machinery in ₹/ha

$X_4$  = Seeds in ₹/ha

$X_5$  = Plant protection in ₹/ha

$X_6$  = Nitrogen in ₹/ha

$X_7$  = Phosphorus in ₹/ha

$X_8$  = Potassium in ₹/ha

Marginal value product of particular resources represented the "expected addition of one unit of that resources while other inputs are held constant" to the marginal factor cost.

$$MVP = b_i \frac{GM(Y)}{GM(X_i)}$$

Where,

$b_i$  = The elasticity of output  $Y$  with respect to input  $X_i$

$GM(Y)$  = Geometric mean of output  $Y$

$GM(X_i)$  = Geometric mean of input  $X_i$

## RESULTS AND DISCUSSION

The present investigation had been undertaken with a view to study "Analysis of Resource Use Efficiency of Gram Production in Gadchiroli District". The major findings of this study are presented under the following heads.

### Resource use efficiency of gram production

Resource use efficiency was worked out through the production function analysis. The production function is often used to determine quantities of inputs that the cultivator uses in production process.

Cobb-Douglas production function was estimated on per hectare basis for the small, medium and large size group of farmers in Table 1.

It is observed from the table 1 that, at overall level, the regression coefficient of human labour and machine charges was significant at one per cent level. Regression coefficient of seeds and nitrogen was significant at five per cent level and regression coefficient of phosphorous was significant at ten per cent level. The regression coefficient of plant protection was negatively non-significant. The positive sign of human labour, machine charges, seeds, nitrogen and phosphorous indicated that one per cent increase in the use of this input could increase the return to the extent of 0.3923, 0.1372,

**Table 1:** Cobb-Douglas production function for gram

Sl. No.	Variable	Small	Medium	Large	Overall
1	Constant (Intercept)	4.3277*** (0.9378)	1.3924 (1.6574)	3.7939*** (1.0854)	1.3384** (0.5244)
2	Coefficient				
(a)	Human labour ( $X_1$ )	0.1936 (0.1634)	0.5925* (0.3272)	0.3586** (0.1227)	0.3923*** (0.1187)
(b)	Bullock labour ( $X_2$ )	0.1104 (0.0695)	-0.1820* (0.0968)	-0.0041 (0.0978)	0.0291 (0.0488)
(c)	Machine charges ( $X_3$ )	0.0009 (0.0677)	-0.0532 (0.0934)	-0.0606 (0.1058)	0.1372*** (0.0449)
(d)	Seeds ( $X_4$ )	-0.2537** (0.1103)	0.4845*** (0.1507)	-0.1394 (0.1307)	0.1489** (0.0748)
(e)	Plant protection ( $X_5$ )	-0.0354 (0.0592)	-0.1345 (0.0995)	-0.0370 (0.1345)	-0.0247 (0.0508)
(f)	Nitrogen ( $X_6$ )	0.1378** (0.0527)	-0.0100 (0.0748)	0.0208 (0.0640)	0.0909** (0.0392)
(g)	Phosphorous ( $X_7$ )	0.1742 (0.1287)	0.0678 (0.1399)	0.1885* (0.0880)	0.1295* (0.0766)
(h)	Potassium ( $X_8$ )	-0.2247 (0.1110)	0.1209 (0.1198)	-0.0355 (0.0729)	0.0674 (0.0563)
3	Coefficient of Determination ( $R^2$ )	0.4585	0.5111	0.5299	0.4152

**Note:** Figures in parentheses indicates standard errors.

\* indicates significant at 10 per cent level of significance; \*\* indicates significant at 5 per cent level of significance; \*\*\* indicates significant at 1 per cent level of significance.



0.1489, 0.0909 and 0.1295 per cent respectively. The negative sign of the regression coefficient of plant protection showed that one per cent additional expenditure on this input would reduce the return of gram by 0.0247 per cent. About 41 per cent of the variation was explained by the variable included in the function.

In small size group, the regression coefficient of seeds was significant at five per cent level. Also nitrogen was significant at five per cent level and other remaining variables were non-significant. About 45 per cent of the variation was explained by the variable included in the function.

In medium size group, the regression coefficient of human labour and bullock labour was significant at ten per cent level, regression coefficient of seeds was significant at one per cent level. Other remaining variables were non-significant. About 51 per cent of the variation was explained by the variable included in the function.

In large size group, the regression coefficient of human labour was significant at five per cent level, regression coefficient of phosphorous was significant at ten per cent level. Other remaining variables were non-significant. About 52 per cent of the variation was explained by the variable included in the function.

It is observed from Table 2 that, at overall level marginal value of product to factor cost ratio of human labour, bullock labour, machine charges, seeds, plant protection, nitrogen, phosphorous and potassium was less than one. This indicates excess use of this inputs. Hence, increasing these inputs are not significant in the crop production.

In small size group, the marginal value of product to factor cost ratio was positive and less than one

in case of human labour, bullock labour, machine charges, nitrogen and phosphorous whereas negative and less than one in case of seeds, plant protection and potassium. This indicates excess use of this inputs.

In medium size group, the marginal value of product to factor cost ratio of human labour, seeds, phosphorous and potassium was positive and less than one whereas of bullock labour, machine charges, plant protection and nitrogen was negative and less than one. This indicates excess use of this inputs. In large size group, the marginal value of product to factor cost ratio of human labour, nitrogen and phosphorous was positive and less than one whereas in case of bullock labour, machine charges, seeds, plant protection, potassium was negative and less than one. This indicates excess use of this inputs so increased level of this inputs cannot increase the production.

### Constraints faced by farmers in production of gram

All the selected gram growers were interviewed for the constraints they are facing in production of gram. The farmers have various constraints like problem of insects and pests, lack of training and demonstrations and problem of wild animals. The constraints faced by farmers were identified and given in Table 3.

It is revealed from the Table 3 that, in the constraints experienced by the farmers in the study area, out of 90 farmers, problem of wild animals was the major problem which was expressed by 79 farmers (87.78 per cent) ranked 1<sup>st</sup> followed by problem of infestation of insects and pests which was expressed by 76 farmers (84.44 per cent) ranked

**Table 2:** Marginal Value of Product at factor cost

Sl. No	Variable	Small	Medium	Large	Overall
1	Human labour ( $X_1$ )	0.2321	0.7126	0.4391	0.4731
2	Bullock labour ( $X_2$ )	0.1577	-0.2571	-0.0058	0.0413
3	Machine charges ( $X_3$ )	0.0013	-0.0728	-0.0800	0.1847
4	Seeds ( $X_4$ )	-0.3489	0.6555	-0.1895	0.2031
5	Plant protection ( $X_5$ )	-0.0558	-0.2097	-0.0561	-0.0385
6	Nitrogen ( $X_6$ )	0.2452	-0.0171	0.0352	0.1578
7	Phosphorous ( $X_7$ )	0.2603	0.1015	0.2827	0.1938
8	Potassium ( $X_8$ )	-0.3672	0.1915	-0.0568	0.1085

**Table 3:** Constraints faced by farmers in production of gram

Sl. No.	Constraints	No. of farmers (out of N=90)	Percentage to total farmers	Rank
1	Problem of Infestation of Insects and pests	76	84.44	II
2	Destruction due to wild animals	79	87.78	I
3	Lack of technical knowledge	67	74.44	IV
4	Non-availability of inputs at proper time	44	48.89	VI
5	Unaware about seed treatment	74	82.22	III
6	Lack of training and demonstrations	58	64.44	V

2<sup>nd</sup>, unaware about seed treatment (82.22 per cent), lack of technical knowledge (74.44 per cent), lack of training and demonstrations (64.44 per cent) and non-availability of inputs at proper time (48.89 per cent) are ranked 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> respectively.

## CONCLUSION

At overall level, the regression coefficient of human labour and machine charges was significant at one per cent level. Regression coefficient of seeds and nitrogen was significant at five per cent level and regression coefficient of phosphorous was significant at ten per cent level. At overall level about 41 per cent of the variation was explained by the variable included in the function. In case of small, medium and large size group about 45 per cent, 51 per cent, 52 per cent variation was explained by the variable included in the function. At overall level marginal value of product to factor cost ratio of all the variables were less than one. This indicate excess use of these inputs. Hence, increasing these inputs

are not significant in the crop production. Major suggestions given by farmers were, availability of pesticides at reasonable prices, awareness and technical knowledge, training and demonstrations about seed treatments methods should be provided.

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# Process Analysis of Production of Fertilizer and Determining Loss due to Failure to Meet the Specification

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## ABSTRACT

The sustainable development is responsible production and efficient use of resources available to human. The present paper refers to the best practices in fertilizer production in improving crop yield, farm profitability and resource efficiency while at the same time reducing adverse environmental impacts. So, the study is based on analysis of performance of four different train in a plant for production of three products namely DAP, N20 and N10 fertilizers by using Exponentially Weighted Moving Average (EWMA) and Moving Average (MA) control charts and Process Capability Indices. Further, the loss to producer due to not meeting the specification limits was determined. The result indicated that most of the time production process is out of control. Due to product failure the organization suffer production loss, time loss as well as money loss. Hence, the producer suffers loss of nutrients and its associated cost and consequently market prices of fertilizers increases which directly affect farmers' pocket. This paper, also, recommended use of Laser Induced Breakdown Spectroscopy (LIBS) technology to give instantaneous quality result for the outgoing product and in turn reducing losses of nutrients, resources, energy and capital.

## HIGHLIGHTS

- ① The organization suffer loss of production, time and money due to product failure resulting in increased market prices of fertilizers.
- ② LIBS technology gives instantaneous quality result for the outgoing product.

**Keywords:** EWMA and MA control charts, Process capability indices, Loss to producer, Specification limits, LIBS

Process analysis is a form of technical writing and expository writing designed to convey to the reader how a change takes place through a series of stages. While the traditional process analysis and a set of instructions are both organized chronologically, the reader of a process analysis is typically interested in understanding the chronological components of a system that operates largely without the reader's direct actions (such as how the body digests an apple), while the reader of a set of instructions intends to use the instructions in order to accomplish a specific, limited task (such as how to bake an apple pie). By contrast, the reader of a mechanism description is more interested in an

object in space (such as the form and nutritional value of a particular kind of apple) (Montgomery 2007).

Control chart is a popular technique that is widely used in statistical process control to identify any possible deviations from a stable state of a process. Shewhart charts are famous for identifying larger shifts, while cumulative sum and exponentially

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weighted moving average control charts are well known for identifying smaller shifts in process parameters (Abbasi *et al.* 2020). It is important for the quality department to control the quality of processes from incoming of raw materials until the finished product. Otherwise, if the rejection and rework processes have taken place, the productivity will be decreased, the main cause of variation can be identified so that the earlier action can be taken in order to overcome and avert similar variation and problem occurred in the future (Bon *et al.* 2011).

Laser-induced breakdown spectroscopy (LIBS) is an atomic spectroscopy technique firstly proposed by Breech and Cross in 1962. It has been praised as the “future super star” in analytical chemistry. Laser-induced breakdown spectroscopy (LIBS) has been widely studied due to its unique advantages such as remote sensing, real-time multi-elemental detection and none-to-little damage. (Guo *et al.* 2021). IFFCO produces 81.5 Lakh Metric Tons of fertilizer in the year 2018-19 of which contribution of Urea fertilizer is 45.6 LMT and that of NPK/DAP fertilizer is 35.9 LMT (Anonymous 2018-19).

A fertilizer is any material of natural or synthetic origin (other than liming materials) that is applied to soils or to plant tissues (usually leaves) to supply one or more plant nutrients essential to the growth of plants. It is classified further according to its chemical compositions. Such as, straight fertilizers which provide a single nutrient (e.g., K, P, or N), the main nitrogen-based straight fertilizer is ammonia or its solutions e.g. Ammonium Nitrate ( $\text{NH}_4\text{NO}_3$ ). Multi-nutrient fertilizers (or complex fertilizers) provide two or more nutrients, for example N and P. Fertilizers are also sometimes classified as inorganic versus organic. Inorganic fertilizers exclude carbon-containing materials except Urea. Organic fertilizers are usually (recycled) plant- or animal-derived matter. They consist of two or more nutrient components and are soluble in water e.g. Diammonium Phosphate (DAP). NPK fertilizers are three-component fertilizers providing nitrogen, phosphorus, and potassium. NPK rating is a rating system describing the amount of nitrogen, phosphorus, and potassium in a fertilizer. NPK ratings consist of three numbers separated by dashes (e.g. 10-10-10 or 16-4-8) describing the chemical content of fertilizers (Anonymous 2021).

## Terminologies

**DAP:** Diammonium Phosphates

**N20:** Ammonium Sulphate having 20% of Nitrogen

**N10:** Ammonium Sulphate having 10% of Nitrogen

Consisting of different amounts of:

TN: Total Nitrogen

$\text{P}_2\text{O}_5$ : Phosphorus Pentoxide

$\text{K}_2\text{O}$ : Potassium Oxide

These fertilizers contain following macronutrients.

N: Nitrogen – Leaf growth;

P: Phosphorus – Development of roots, flowers, seeds, fruit;

K: Potassium – Strong stem growth, movement of water in plants, promotion of flowering and fruiting.

## Objective

The main objective of this study is to analyze the production process of fertilizer to determine loss due to failure to meet the specification.

## MATERIALS AND METHODS

### Data Collection

Primary data was collected in an excel spreadsheet. There was one production plant having 4 sub plants (train).

Data consists of 4 train namely A, B, C and D.

In train A and B, three products are produced namely DAP, N20, N10.

In train C and D, two products are produced namely DAP, N20.

Each train is working independently. It is not fixed for a train to produce same particular product. There is different length of run of a particular product, which is also not fixed as it depends upon the amount of the input chemicals. Usually, after DAP, N20 or N10 are produced for, the remaining Nitrogen of DAP is used for producing N20 or N10. Standard ratio of nutrients in fertilizers are as follows:

- ♦ In DAP, the ratio of N and  $\text{P}_2\text{O}_5$  are 18:46 in percentage.





- ♦ In N20, the ratio of N and P<sub>2</sub>O<sub>5</sub> are 20:20 in percentage.
- ♦ In N10, the ratio of N, P<sub>2</sub>O<sub>5</sub> and K are 10:26:26 in percentage.

The above ratios of nutrients are the target mean and the specification limits are (target mean) (Anonymous 2021). Products not meeting lower specification limits are recycled in the plant.

After production, samples were sent to the quality control department. After sampling inspection, the reports of ratios of nutrient is given to the person responsible to data entry. Hence, the report is delayed by 1 hour, resulting in delay of remedial action. Note that the numeric values in the data are sample mean and range is not provided.

### STATISTICAL ANALYSIS

The MA and EWMA control chart were applied to draw conclusion about production process and to determine the loss using MS-Excel and R-Programming software (Version 3.3.3).

#### Moving Average Control Charts

The moving average chart is control chart for the mean that uses the average of the current mean and a handful of previous means to produce each moving average. This chart is used to monitor the mean of a process based on samples taken from the process at given times (hours, shifts, days, weeks, months, etc.). The measurements of the samples at a given time constitute a subgroup. The moving average chart relies on the specification of a target value and a known or reliable estimate of the standard deviation. For this reason, the moving average chart is better used after process control has been established.

Suppose sample of size  $n$  are collected from the process. Let the first  $t$  sample mean be denoted by  $\bar{X}_1, \bar{X}_2, \dots, \bar{X}_t$ . The moving average of width  $w$  at time step  $t$  is given by;

$$M_t = \frac{\bar{X}_t + \bar{X}_{t-1} + \dots + \bar{X}_{t-w+1}}{w} \quad \dots(1)$$

The center line and control limits for the moving average chart are given by;

$$\left. \begin{aligned} CL &= \bar{\bar{X}} \\ UCL &= \bar{\bar{X}} + 3 \frac{\sigma}{\sqrt{nw}} \\ LCL &= \bar{\bar{X}} - 3 \frac{\sigma}{\sqrt{nw}} \end{aligned} \right\} \dots(2)$$

From eq. (2), it can be seen that as  $w$  increases, the width of the control limits decreases. So, to detect shift of smaller magnitudes, larger value of  $w$  should be chosen.

#### EWMA Control Chart

An EWMA control chart is a time-weighted control chart that plots the exponentially weighted moving averages. EWMA charts are especially suited to monitor processes that exhibit a drifting mean over time, or for detecting small shifts in a process. The EWMA is a statistic for monitoring the process that averages the data in a way that gives less and less weight to data as they are further removed in time. One of the advantages of EWMA chart over MA chart is that the former is more effective in detecting changes in process parameter and it is given by;

$$G_t = r\bar{X}_t + (1-r)G_{t-1}$$

Where  $r$  is a weighting constant ( $0 < r \leq 1$ ).

If sample means  $\bar{X}_1, \bar{X}_2, \dots, \bar{X}_{t-1}$ , are assume to be independent of each other. The upper and lower control limits are;

$$\begin{aligned} UCL &= \bar{\bar{X}} + 3\sigma \sqrt{\frac{r}{(2-r)n}} \\ LCL &= \bar{\bar{X}} - 3\sigma \sqrt{\frac{r}{(2-r)n}} \end{aligned}$$

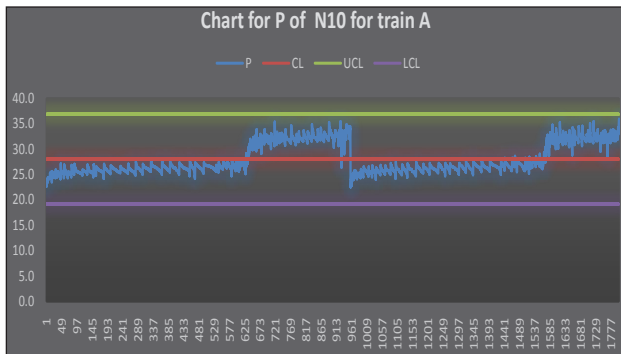
An EWMA control chart is based on a concept similar to that of a moving average chart. By choosing an adequate set of weights. However, where recent sample means are more heavily weighted. The ability to detect small changes in process parameter is increased. If the weighting factor  $r$  is selected as;

$$r = \frac{2}{w+1}$$

Where  $w$  is moving average span, then the moving average method and EWMA method are equivalent. There are guidelines for choosing the value of  $r$ . If the goal is to detect small shift in process parameters as soon as possible, use a small value of  $r$  (say 0.1). If  $r = 1$  is used, the EWMA chart reduces to the standard Shewhart control chart for mean.

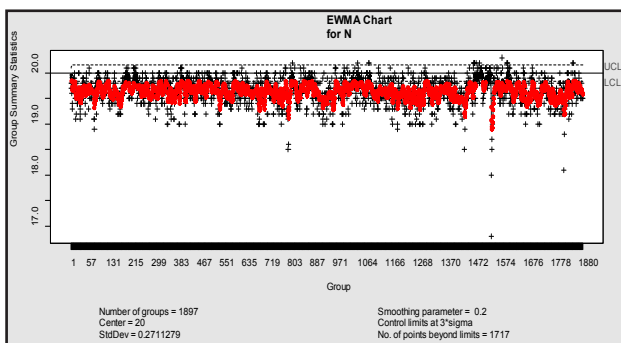
### Analyzing procedure

The data was cleaned using filter in MS-Excel for, sometimes sub plants were shutdown and recycling of non-conforming products (failing to meet the specified criteria and guidelines). Then the data is filtered sub plant wise, product wise and nutrient wise for further analysis and excess amount of chemicals in the outgoing product is determined. For a rough idea about the production ( $\bar{X}, s$ )- chart is constructed using sample standard deviation.



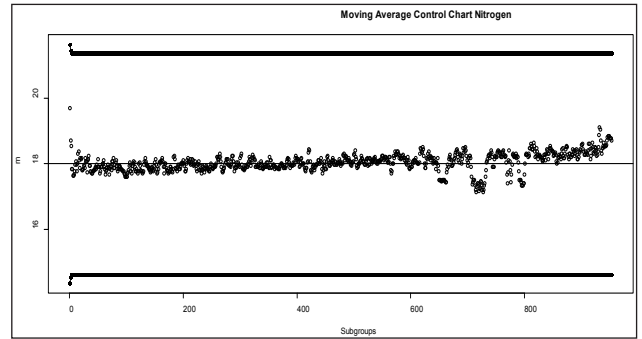
Though the points are within control limits in the above chart but there is pattern visible and this include non-confirming items which are then recycled so the data was cleaned for determining loss.

### Applying EWMA Control Chart



Above chart shows that the process is not centered around the target mean while red points suggesting that the 1717 points out of 1897 points lie below the LCL and + sign showing the actual data value.

### Applying MA Control Chart



Moving average control chart for N of N20 of plant A

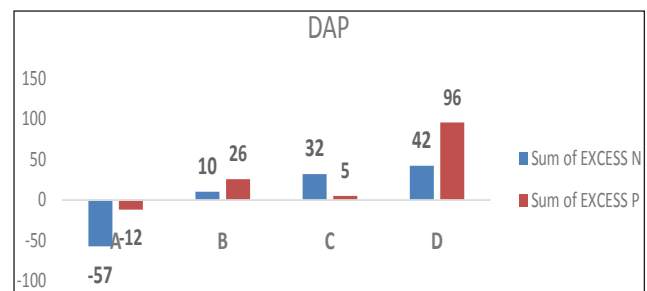
The above MA control chart shows that all the points are under control. Period of 3 hours is used for calculating moving averages.

### RESULTS AND DISCUSSION

As shown in Table 1 and Fig. 1, excess nutrient of N and  $P_2O_5$  for DAP in all the 4 sub plants is calculated and multiplied by tons of amount produced in an hour which is 45 tons per hour for both N and  $P_2O_5$ .

**Table 1:** Excess nutrient of N and  $P_2O_5$  for DAP in sub plants A, B, C and D

Sub Plant	Sum of EXCESS N	Sum of EXCESS P
A	-57.3795	-12.1005
B	10.0485	25.695
C	31.905	5.085
D	42.21	95.58
<b>Grand Total</b>	<b>26.784</b>	<b>114.2595</b>

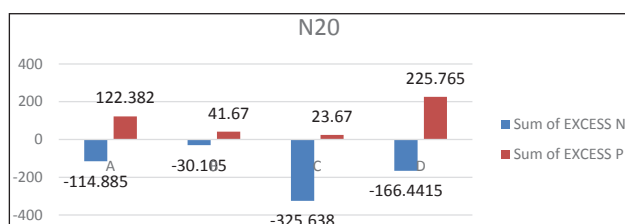


**Fig. 1:** Excess nutrient of N and  $P_2O_5$  for DAP in sub plants A, B, C and D

As shown in Table 2 and Fig. 2, excess nutrient of N and  $P_2O_5$  for N20 in all the 4 sub plants is calculated and multiplied by tons of amount produced in an hour which is 45 tons per hour for both N and  $P_2O_5$ .

**Table 2:** Excess nutrient of N and P<sub>2</sub>O<sub>5</sub> for N20 in sub plants A, B, C and D

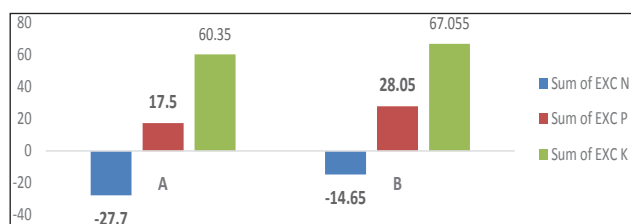
Sub Plant	Sum of excess N	Sum of excess P
A	-114.885	122.382
B	-30.105	41.67
C	-325.638	23.67
D	-166.4415	225.765
<b>Grand Total</b>	<b>-637.0695</b>	<b>413.487</b>


**Fig. 2:** Excess nutrient of N and P<sub>2</sub>O<sub>5</sub> for N20 in sub plants A, B, C and D

As shown in Table 3 and Fig. 3, excess nutrient of N and P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O for N10 in sub plants A and B is calculated and multiplied by tons of amount produced in an hour which is 50 tons per hour for both N and P<sub>2</sub>O<sub>5</sub>.

**Table 3:** Excess nutrient of N and P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O for N10 in sub plants A and B

Row Labels	Sum of excess N	Sum of excess P	Sum of excess K
A	-27.7	17.5	60.35
B	-14.65	28.05	67.055
<b>Grand Total</b>	<b>-42.35</b>	<b>45.55</b>	<b>127.405</b>


**Fig. 3:** Excess nutrient of N and P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O for N10 in sub plants A and B

### Total loss

Determining loss to the company for not meeting the guidelines:

- ♦ Nutrient loss of N in all the three fertilizers.
- ♦ In DAP, total failure cases are 435 resulting in loss of -126 tons of nitrogen.

- ♦ In N20, total failure cases are 143 resulting in loss of -1013 tons of nitrogen.
- ♦ In N10, total failure cases are 10481 resulting in loss of 516 tons of nitrogen.
- ♦ Nutrient loss of P<sub>2</sub>O<sub>5</sub> in all the three fertilizers:
- ♦ In DAP, total failure cases are 1718 resulting in loss of 496.5 tons of P<sub>2</sub>O<sub>5</sub>.
- ♦ In N20, total failure cases are 1001 resulting in loss of 183.8 tons of P<sub>2</sub>O<sub>5</sub>.
- ♦ In N10, total failure cases are 1621 resulting in loss of 1144 tons of P<sub>2</sub>O<sub>5</sub>.
- ♦ Nutrient loss of K in N10 fertilizers:
- ♦ In N10, total failure cases are 657 resulting in loss of 200.4 tons of potassium.

**Table 4:** Calculation of Total Loss

	N10	DAP	N20	Total in tons	Rates in USD/ton**	Total USD
Nitrogen	516	-126	-1013	-623	550	*
P <sub>2</sub> O <sub>5</sub>	183.8	496.5	1144	1824.3	130	2, 37, 159
Potassium	200.4	_	_	200.4	265	53, 106
Total in USD						2, 90, 265
INR/USD						69
INR						2, 00, 28, 285
INR (in crores)						2.00

\*As nitrogen in total tons is -623, which indicate lack of nutrient N in the final product;

\*\*Sources: <https://aei.ag/tag/2019-fertilizer-prices/> and <https://ycharts.com/>.

### CONCLUSION

In the light of above discussion, it is observed that most of the time production process is out of control. Due to product failure, organization suffer production loss, time loss and money loss. Due to delay of one-hour, the remedial action taken is also delayed and in that entire span machines continued to produce non-conforming products. Hence, the producer suffers loss of nutrients and its associated cost. Consequently, the prices of fertilizers go up as excess raw materials are used in the production resulting in higher prices to be paid by farmers. Also, more than optimum levels of fertilizers in the soil affect negatively to the environment and ecology. Therefore, it is recommended to use new quality



control techniques by the organization for online results of nutrient count in the output fertilizer. For example, metallurgy, coal, etc. industries are using LIBS technology for *in situ* or real time quality result which costs around 4 crore which can be incurred from the total loss and company can recover this amount within few years.

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# Morphological Characterization of Roselle (*Hibiscus sabdariffa* L.) Germplasm for Qualitative Traits

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## ABSTRACT

An experiment has been conducted with an objective to study qualitative characters in roselle for two years at three different Agro-climatic zones of India (two of West Bengal and third in Andhra Pradesh) during *Kharif*, 2013 & 2014 from a set of sixty roselle germplasm consisting of forty five indigenous, eleven exotic and four released varieties. Data on eighteen qualitative traits *viz.*, stem pigmentation, nature of stem, leaf shape, leaf margin colour, leaf vein colour, leaf angle, lobe number, petiole colour, nature of petiole, calyx pigmentation, calyx type, nature of calyx, petal colour, petal eye spot, pollen colour, staminal column colour, stigma colour and pod shape have been recorded. Five traits *viz.*, stem pigmentation, nature of stem, petiole pigmentation, nature of petiole and calyx pigmentation have three types of variants (polymorphism), whereas, the rest of the traits showed two types of variants (dimorphism). Roselle germplasm can be divided based on stem pigmentation and leaf shape. Stem pigmentation, leaf shape and petiole colour are the most variable traits for fibre crop, whereas, calyx pigmentation, petal eye spot and pod shape are most variable traits for calyx and seed crop. Characterization of germplasm on the basis of their qualitative traits will be useful in identification of genotypes for different purposes including framing of DUS characteristics in roselle.

## HIGHLIGHTS

- Sixty genotypes of roselle were characterized for 18 qualitative traits.
- Study was conducted at three agro-climatic zones, two in West Bengal and one in Andhra Pradesh.
- The study will be useful for framing DUS characteristics in Roselle.

**Keywords:** Characterization, Germplasm, Qualitative traits, Roselle

Roselle belongs to the family *Malvaceae* which consists of more than 100 known species of distinct natural group, many of which are handsome ornamental with large, showy, delicate flowers. Roselle is mainly used for fibre, food and medicinal purpose along with kenaf (*Hibiscus cannabinus* L.) (Mohamed and El Gabri 2013). *Hibiscus sabdariffa* was an allotetraploid species ( $2n = 4x = 36$ ) which occurs only in a state of domestication (Cruz and Solano 2013). Roselle has been cultivated extensively in India, Egypt, Senegal and Thailand for its red

calyxes, which are used for making jams, gelatins and beverages.

Raw jute includes the fibre obtained from two species of jute *Corchorus olitorius* L. (tossa jute) and *Corchorus capsularis* L. (white jute) and two species

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of mesta, *Hibiscus sabdariffa* L. (roselle) and *Hibiscus cannabinus* L. (kenaf) occupying only 0.55% of cropped area, provide livelihood to 5 million people in farming, industry and trade involving 4 million farm families, 0.25 million industrial workers and 0.5 million traders in India. In India, roselle is one of the most important bast fibre crop which occupies second place in area and production after jute (Hariram and Appalaswamy 2014).

Morphological characterization is the foremost step for classification and evaluation of the germplasm of a crop (Smith and Smith 1989). Characterization of germplasm in any crop increases their utility in crop breeding program. The use of morphological traits is the most common approach utilized to estimate relationships between genotypes (Rao *et al.* 2021). The conservation and characterization of these genetic resources is a necessity not only for posterity, but also for utilization in different crop improvement programs such as yield improvement and resistance or tolerance to biotic / abiotic stresses. The concept of distinctness, uniformity and stability (DUS) are fundamental to the characterization of a variety as a unique creation. The uniqueness of a particular variety is to be established by DUS test. The first step to implement PPV&FR Act provisions is formulation of national test guidelines for conducting DUS tests. Till date very little work was done on morphological characterization in roselle with few traits only. In this context, an attempt was made to characterize a set of 60 genotypes of roselle including indigenous, exotic and released varieties for eighteen morphological traits to identify the morphological variability present within the roselle germplasm for qualitative traits.

## MATERIALS AND METHODS

Sixty roselle (*Hibiscus sabdariffa* L.) genotypes consisting of eleven exotic lines, four released varieties and 45 indigenous accessions were evaluated at three different agro-climatic environments viz., North Coastal zone, Andhra Pradesh at Agricultural Research Station, Ragolu (Latitude 18° 24' N; Longitude 83. 84° E at an altitude of 27m above mean sea level); Indo-Gangetic zone, West Bengal at Instructional Farm, Bidhan Chandra Krishi Vishwavidyalaya, Jaguli (Latitude 22° 93' N; Longitude 88. 59° E at an altitude of 9.75m above mean sea level) for first year and at Teaching farm,

Mondaury, BCKV (Latitude 22° 87' N; Longitude 88. 59° E at an altitude of 9.75 m above mean sea level) for second year and Terai Zone, West Bengal at University Farm, Uttar Banga Krishi Vishwavidyalaya (Latitude 26° 19' N; Longitude 89. 23° E at an altitude of 43 m above mean sea level). The experiments were sown during early *kharif* seasons in 2013 and 2014 at the above three zones. The experimental trial was laid out in randomized block design in two replications with a plot size of four rows of 2m length with intra-row spacing of 10 cm and inter-row spacing of 30 cm accommodating 20 plants in a row. Recommended package of practices was followed to raise a good crop. Observations on eighteen qualitative traits viz., stem pigmentation, nature of stem, leaf shape, leaf margin colour, leaf vein colour, leaf angle, lobe number, petiole colour, nature of petiole, calyx pigmentation, calyx type, nature of calyx, petal colour, petal eye spot, pollen colour, staminal column colour, stigma colour and pod shape have been recorded on plot basis in all the three locations. These characters were recorded in different growth stages of crop viz., vegetative, flowering, pod formation stage and maturity stage.

## RESULTS AND DISCUSSION

The observations of eighteen qualitative traits recorded from the three locations were found to be stable without any deviation for any of the character under study which proves that there is no influence of environment on qualitative characters. Data on eighteen qualitative traits in roselle were presented in Table 1 (a and b) and detailed trait-wise classification is given below:

- ♦ **Stem pigmentation:** This character showed polymorphism with three types of variations i.e. green, green with nodal pink and pink colour. Nine genotypes had green stem, thirty two genotypes with green bearing nodal pigmentation and nineteen genotypes of pink pigmentation.
- ♦ **Nature of stem:** This character was found to be polymorphic with three different types such as smooth, hairy and spiny. The stems were smooth in ten genotypes, hairy in twenty one genotypes and twenty nine were spinous.
- ♦ **Leaf shape:** This character was found dimorphic with fully lobed and partially lobed leaves. Fifty

Table 1a: Characterization of sixty roselle genotypes based on eighteen qualitative characters

Genotype	Stem			Leaf			Petiole			Calyx			Petal		Pollen colour	SCC	Stigma colour	Pod shape
	Pig.	Nature	Shape	Margin colour	Vein colour	No. of lobes	Angle	Colour	Nature	Pig.	Type	Nature	Colour	Eye spot				
AR-14	P	H	LL	Pig.	P	5	Ho.	P	H	FP	F	LH	Y	Pr.	Br.	DR	O	
AR-19	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AR-42	GNP	H	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	
AR-45	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AR-48	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AR-50	G	H	LL	NO	G	5	SE	G	SP	NP	N	H	Y	Ab.	Y	White	R	
AR-55	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AR-66	GNP	H	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	
AR-67	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AR-71	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AR-79	GNP	H	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	
AR-80	P	H	LL	Pig.	P	5	Ho.	P	H	FP	N	H	SRP	Pr.	Br.	DR	R	
AR-81	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AR-85	P	H	LL	Pig.	P	5	Ho.	P	H	FP	N	H	Y	Pr.	Br.	DR	O	
AR-88	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
R-16	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
R-29	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
R-30	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
R-37	GNP	H	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	
R-48	G	H	PLL	Pig.	G	3	Ho.	G	SP	NP	N	H	Y	Ab.	Y	White	R	
R-67	P	H	LL	Pig.	P	5	Ho.	P	H	FP	N	H	Y	Pr.	Br.	DR	O	
R-68	G	SM	LL	NO	G	5	Ho.	G	SP	NP	N	H	Y	Ab.	Y	White	O	
R-77	G	SM	LL	NO	G	5	Ho.	G	SP	NP	N	H	Y	Ab.	Y	White	O	
R-86	GNP	SP	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	
R-134	G	SP	PLL	NO	G	3	SE	G	SP	NP	N	H	Y	Ab.	Y	White	O	
R-180	G	SP	LL	NO	G	5	Ho.	G	SP	NP	N	H	Y	Ab.	Y	White	R	
R-225	GNP	SP	LL	Pig.	P	5	SE	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
R-243	P	H	LL	Pig.	P	5	Ho.	P	H	FP	N	H	SRP	Pr.	Br.	DR	R	
R-271	GNP	H	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	
R-284	GNP	H	LL	Pig.	P	5	SE	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	

Pig. – Pigmentation; P – Pink; GNP – Green with nodal pink; G – Green; H – Hairly; SP – Spiny; SM – Smooth; LL – Lobed leaf; PLL – Partially lobed leaf; Ho. – Horizontal; S E – Semi-erect; EP – Edges pink; FP – Fully pigmented; PP – Partially pigmented; NP – No pigmentation; F – Fleahy; N – Normal; LH – Less hairy; Y – Yellow; SRP – Slightly rose pigmented; Pr. – Present; Ab. – Absent; Br. – Brown; SCC – Staminal column colour; DR – Dark red; LG – Light green; O – Oval and R-Round

**Table 1b:** Characterization of roselle genotypes based on eighteen qualitative characters

Genotype	Stem			Leaf			Petiole			Calyx			Petal		Pollen colour	SCC	Stigma colour	Pod shape
	Fig.	Nature	Shape	Margin colour	Vein colour	No. of lobes	Angle	Colour		Nature	Type	Nature	Colour	Eye spot				
								Colour	Nature									
R-318	P	H	LL	Pig.	P	5	Ho.	P	H	FP	N	H	SRP	Pr.	Br.	DR	O	
R-322	GNP	H	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	
ER-56	G	SP	LL	NO	G	5	Ho.	G	SP	NP	N	H	Y	Ab.	Y	White	R	
ER-57	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
ER-60	GNP	SM	PLL	Pig.	P	5	SE	EP	SM	PP	F	LH	Y	Pr.	Br.	DR	R	
ER-68	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AS-80-6	P	H	LL	Pig.	P	5	Ho.	P	H	FP	F	LH	Y	Pr.	Br.	DR	R	
AS-80-7	GNP	H	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	R	
AS-80-19	P	SM	LL	Pig.	P	5	Ho.	P	SM	FP	N	LH	SRP	Pr.	Br.	DR	R	
AS-80-26	P	SM	LL	Pig.	P	3	SE	P	SM	FP	N	LH	Y	Pr.	Br.	DR	R	
AS-80-29	G	SP	PLL	NO	G	5	SE	G	SP	NP	N	H	Y	Ab.	Y	White	O	
AS-81-1	P	SM	PLL	Pig.	P	5	Ho.	P	SM	FP	F	LH	Y	Pr.	Br.	DR	O	
AS-81-2	P	SM	LL	Pig.	P	5	Ho.	P	SM	FP	F	LH	Y	Pr.	Br.	DR	R	
AS-81-3	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	O	
AS-81-5	P	H	LL	Pig.	P	5	Ho.	P	H	FP	N	H	Y	Pr.	Br.	DR	R	
AS-81-9	P	SP	LL	Pig.	P	5	Ho.	P	SP	FP	N	H	Y	Pr.	Br.	DR	R	
AS-81-14	GNP	H	LL	Pig.	P	5	Ho.	EP	H	PP	N	H	Y	Pr.	Br.	DR	O	
AS-81-17	P	SM	LL	Pig.	P	5	Ho.	P	SM	FP	N	LH	Y	Pr.	Br.	DR	O	
AS-81-22	P	SM	PLL	Pig.	P	5	Ho.	P	SM	FP	F	LH	Y	Pr.	Br.	DR	O	
REX-6	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
REX-14	P	SP	LL	Pig.	P	5	Ho.	P	SP	FP	N	H	Y	Pr.	Br.	DR	R	
REX-34	GNP	SM	PLL	Pig.	P	5	Ho.	EP	SM	PP	F	LH	Y	Pr.	Br.	DR	R	
REX-38	GNP	SP	PLL	Pig.	P	5	SE	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
REX-45	G	H	PLL	NO	G	5	Ho.	G	H	NP	N	H	Y	Ab.	Y	White	O	
REX-52	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	O	
REX-63	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
HS-4288	GNP	SP	LL	Pig.	P	5	Ho.	EP	SP	PP	N	H	Y	Pr.	Br.	DR	R	
AMV-4	P	SP	LL	Pig.	P	5	Ho.	P	H	FP	N	H	Y	Pr.	Br.	DR	O	
AMV-5	P	H	LL	Pig.	P	5	Ho.	P	H	FP	N	H	Y	Pr.	Br.	DR	O	
AMV-7	P	SP	LL	Pig.	P	5	Ho.	P	H	FP	N	H	Y	Pr.	Br.	DR	O	

*Pig.* – Pigmentation; *P* – Pink; *GNP* – Green with nodal pink; *G* – Green; *H* – Hair; *SP* – Spiny; *SM* – Smooth; *LL* – Lobed leaf; *PLL* – Partially lobed leaf; *Ho.* – Horizontal; *SE* – Semi-erect; *EP* – Edges pink; *FP* – Fully pigmented; *PP* – Partially pigmented; *NP* – No pigmentation; *F* – Fleahy; *N* – Normal; *LH* – Less hairy; *Y* – Yellow; *SRP* – Slightly rose pigmented; *Pr.* – Present; *Ab.* – Absent; *Br.* – Brown; *SCC* – Staminal column colour; *DR* – Dark red; *LG* – Light green; *O* – Oval and R-Round.





one genotypes were fully lobed type and nine genotypes were partially lobed type.

- ♦ **Leaf margin colour:** This character showed dimorphism with two distinct morphology, without and with pigment leaf margin. Nine genotypes were devoid of pigmentation, whereas, fifty one genotypes were pigmented.
- ♦ **Leaf vein colour:** Dimorphism with two types of leaf vein colour i.e. pink and green was noticed. Fifty one genotypes were with pink and nine genotypes with green leaf vein were detected.
- ♦ **Leaf lobe number:** Dimorphism with two types of variations i.e. five and three lobed types was evident for the character. Fifty seven genotypes were five lobed type and rest were of three lobed type.
- ♦ **Leaf angle:** This character showed dimorphism with horizontal and semi-erect angle of leaves. Horizontal leaf angle was found in fifty two genotypes and rest were semi-erect type.
- ♦ **Petiole colour:** This character showed polymorphism with green, pink edged and pink pigmented types. Nine genotypes were green type, thirty two genotypes were pink edged and nineteen genotypes were pink.
- ♦ **Nature of petiole:** This character showed polymorphism with three distinct types i.e. smooth, hairy and spiny. Eight genotypes were smooth type, twenty two were hairy and thirty with spiny petioles.
- ♦ **Calyx pigmentation:** This character showed polymorphism with three types of variations i.e. green, partially pigmented and fully pigmented. Nine genotypes were green, thirty two genotypes were partially pigmented and nineteen genotypes were fully pigmented.
- ♦ **Type of calyx:** This character showed dimorphism with two types of variations i.e. normal and fleshy calyx of which fifty three were normal type and rest were fleshy type.
- ♦ **Nature of calyx:** Dimorphism was highlighted for the character with hairy and less hairy types. Fifty and ten genotypes were found hairy and less hairy, respectively.
- ♦ **Petal colour:** Dimorphism with two types of variations i.e. yellow and light red petal colour

were evident in the genotypes of which fifty six had yellow and rest with light red petal colour.

- ♦ **Petal eye spot:** Variation among genotypes for the character were found dimorphic as presence or absence of petal eye spot. Fifty one genotypes were having petal eye spot which was conspicuously absent in nine genotypes.
- ♦ **Pollen colour:** This character showed dimorphism with two types of variations i.e. brown and yellow types. Fifty one genotypes were brown type and nine genotypes were yellow type.
- ♦ **Staminal column colour:** This character showed dimorphism with two distinct types as red and white. Fifty one genotypes were having red coloured while rest were with white staminal column.
- ♦ **Stigma colour:** Dimorphism with dark red and light green coloured stigma was evident of which fifty one were dark red and rest with light colour.
- ♦ **Pod shape:** Dimorphism with round and oval types were highlighted for the character and fifty one genotypes were with round and rest with oval shaped pods.

Omalsaad *et al.* (2012) studied four qualitative morphological traits viz., stem colour, leaf shape, flower colour and fruit colour in nine roselle accessions and reported similar type of variations. Mohamed and El Gabri (2013) collected 126 accessions of roselle from Sudan and were characterized for their morpho-agronomical characters and observed considerable variation within and between accessions for both qualitative and quantitative characters such as growth habit, leaf, stem, flower and calyx characters. Cruz and Solano (2013) characterized 47 accessions of roselle and reported that leaf colour, earliness, stem colour, colour of bracts and presence of glands were the most important qualitative characters influenced for variation in the genotypes. Falusi *et al.* (2014) collected six accessions of roselle from different parts of Nigeria and found four different types of colour of calyx (red, light red, deep red and green) which indicated that there is a store of genetic variation that can be exploited for improvement of roselle. Seyyed and Mansoor (2017) studied four



qualitative morphological traits viz., stem colour, leaf shape, flower colour and calyx colour in two roselle varieties.

## CONCLUSION

In general, roselle is mainly used for its fibre and calyx purpose, genotypes with more plant height are used for fibre purpose, whereas, shorter genotypes were used for calyx and seed purpose. Roselle genotypes can be grouped based on stem pigmentation and leaf shape. Stem pigmentation, leaf shape and petiole colour are the most variable traits for fibre crop, whereas, calyx pigmentation, petal eye spot and pod shape are most variable traits for calyx and seed crop. Characterization of germplasm on the basis of their qualitative traits will be useful in identification of genotypes for different purposes including framing of DUS characteristics in roselle.

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# Effect of Moisture Stress on Wheat Crop by IW/CPE Approach on Water Requirement and Water Use Efficiency

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## ABSTRACT

Wheat is one of the most important cereal crops and staple food of nearly 35 percent of the world population. In climatologically approaches, irrigation is scheduled on IW/CPE ratio. In IW/CPE approach, known amount of irrigation water is applied when cumulative pan evaporation reaches predetermined level. The experiment was conducted in randomized block design with irrigation scheduling on climatological approach i.e. on IW/CPE ratios of IW/CPE = 0.6, IW/CPE = 0.8, IW/CPE = 1.0, IW/CPE = 1.2 and control treatment with six irrigations at critical growth stages of wheat. Seasonal water requirement of wheat was found to be highest (570 mm) under irrigation scheduling at control treatment ( $I_4$ ). Favorable soil moisture was maintained in the irrigation scheduling treatment of IW/CPE = 1.2 ( $I_4$ ) throughout the growing period and it was always maintained in allowable depletion regime. However, soil moisture was inadequate in irrigation scheduling at IW/CPE = 0.6 ( $I_1$ ). Highest water use efficiency was recorded in treatment  $I_2$  which may due to lowest water use, followed by  $I_3$ ,  $I_4$ ,  $I_1$  and  $I_5$ . Irrigation scheduling at IW/CPE = 1.2 ( $I_4$ ) recorded highest grain yield of wheat but it was at par with  $I_3$ . Grain yield recorded in treatment  $I_3$  (IW/CPE = 1.0) was significantly higher than that in treatment  $I_5$  (Control) with saving of water of 13 %.

## HIGHLIGHTS

- ① Seasonal water requirement of wheat was found to be highest (570 mm) under irrigation scheduling at  $I_4$ .
- ① Highest saving of water 38 % was achieved in  $I_1$  (IW/CPE=0.6)
- ① Favourable soil moisture was maintained in the irrigation scheduling treatments IW/CPE = 1.2 ( $I_4$ ) throughout the growing period.
- ① Highest water use efficiency (0.64) was recorded in treatment  $I_2$ .
- ① Irrigation scheduling at IW/CPE = 1.2 ( $I_4$ ) recorded highest grain yield of wheat.

**Keywords:** Irrigation Scheduling, IW/CPE, Water requirement, Water use efficiency, Soil Moisture

Wheat (*Triticum aestivum* L.) is the first important and strategic cereal crop for the majority of world's populations. Efficient water management requires a thorough study of plant water relationship, climate, agronomic practices and economic assessment. In Wheat, different growth stages such as crown root initiation, tillering, late jointing, boot flowering, milk and dough could be well delineated. Experiments conducted to study the important stages critical in their demand for water have clearly indicated that some stages can tolerate moisture stress to a certain extent. Irrigation scheduling is the systematic method by which producer can decide

on when to irrigate and how much water to apply. The goal of effective scheduling programs is to supply the plants with sufficient water while minimizing losses to deep percolation or runoff. Irrigation scheduling depends on soil, crop, atmospheric, irrigation systems and operational factors. Irrigation scheduling techniques can be

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based on soil water depletion approach, plant basis or plant indices, climatic approaches, critical growth stage approaches and plant water status itself. In soil water depletion approaches, the available soil moisture in the root zone is a good criterion for scheduling irrigation. When the soil moisture in a specified root zone depth is depleted to a particular level (which is different for different crops) it is too replenished by irrigation. In plant basis or plant indices, as the plant is the user of water, it can be taken as a guide for scheduling irrigation. In critical growth approaches, irrigation scheduling at growth stage of crop at which moisture stress level reaches to irrevocable yield loss. These stages are known as critical period or moisture sensitive period. In plant water status approaches, water content in plant itself is considered for scheduling irrigation however, it is yet common use for wants of standard low cost techniques. Whereas, in climatological approaches, the amount of water lost by evapotranspiration is estimated from climatological data. When ET reaches in a particular level, irrigation is scheduled. The amount of irrigation given is either equal to ET or fraction of ET. Different methods of climatic approaches are IW/CPE ratio method and pan evaporation method. In IW/CPE approach, known amount of irrigation water is applied when cumulative pan evaporation reaches predetermined level. For practical purpose, irrigation should be started when allowable depletion of available moisture in the root zone reaches. The available water is soil moisture which lies between field capacity and permanent wilting point.

Thus, irrigation scheduling provides information to the managers to develop irrigation strategies for each plot of field on the farm. Keeping these points in view, experiment will be conducted to assess the water need of wheat crop throughout the growing season using different levels of IW/CPE ratio, by determining the irrigation interval, number of irrigations, moisture depletion pattern.

## MATERIALS AND METHODS

A field experiment on "Performance of wheat under different IW/CPE ratio" was conducted during winter at the Farm of Wheat Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The climate of Akola is subtropical semi-arid characterized by three distinct seasons namely

summer becoming hot and dry from March to May. The warm and rainy monsoon fall from June to Oct. and winter with mild cold from November to Feb. Akola is situated in the subtropical zone at the latitude of 20° 42' North and longitude of 77° 02' East. Altitude of the place is 307.41 m above the mean sea level and average annual precipitation is 750 mm. The normal mean monthly maximum temperature during the hottest month (May) is 42 °C while the normal mean monthly minimum temperature in the coldest month (December) is 10.7 °C. The mean daily evaporation reaches as high as 19.0 mm in the month of May and as low as 3.00 mm in the month of August. The physical and chemical properties are determined are showed in Table 1.

**Table 1:** Mechanical-chemical composition of experimental soil

Sl. No.	Particulars	Values	Analytical method used
<b>(A) Mechanical composition</b>			
1	Sand (%)	14.78	
2	Silt (%)	33.69	Bouyous hydrometer method
3	Clay (%)	51.53	
4	Textured class	Clayey	
<b>(B) Chemical composition</b>			
1	Soil organic carbon (%)	0.63	Walkyey and Black method
2	Available nitrogen (kg/ha)	238.3	Modified Kjeldahl Method
3	Available phosphorus (kg/ha)	14.6	Olsen's Method
4	Available potassium (kg/ha)	266.0	Flame photometric
5	pH	7.84	Beckman's Glass electrode pH meter
6	EC (ds/m)	0.77	Conductivity bridge from 1:2:5 soil water ratio
<b>(C) Single value soil moisture constant</b>			
1	Bulk density (gm/cm <sup>3</sup> )	1.18	Core sampler method
2	Field Capacity (%)	38.25	Pressure plate and pressure membrane apparatus
3	Permanents wilting point (%)	17.21	Pressure plate and pressure membrane apparatus

## Experimental details

The field experiment was laid out in randomized

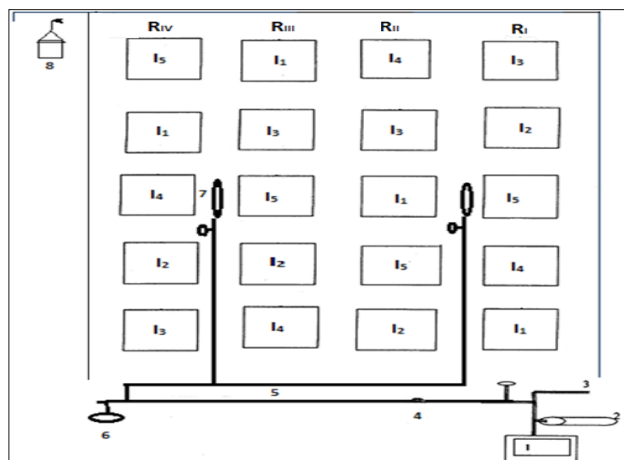
block design, with four replication and five treatments. In four treatments out of five, irrigation was scheduled on the basis of various IW/CPE ratios and in one control treatment irrigation was scheduled at critical growth stages of wheat. The details of treatments are given in Table 2.

**Table 2:** Treatment details

Treat.	Treatment Details
I <sub>1</sub>	IW/CPE ratio = 0.6
I <sub>2</sub>	IW/CPE ratio = 0.8
I <sub>3</sub>	IW/CPE ratio = 1.0
I <sub>4</sub>	IW/CPE ratio = 1.2
I <sub>5</sub>	Control with six irrigations at Crown Root Initiation (CRI), Maximum Tillering, Late Jointing, Flowering, Milking Stage and Dough Stage.

**Table 3:** Experimental Details

Sl. No.	Particulars	Specification
1	Crop	Wheat
2	Scientific name	<i>Triticum aestivum</i> L.
3	Variety	AKAW – 4627
4	Experimental design	Randomized Block Design
5	Number of replications	Four
6	Number of treatments	Five
7	Number of plots	20
8	Plot size	6m × 1.8m
9	Inter-space between replications per plots	2 m
10	Season	Winter 2012
11	Crop spacing	18 cm (row to row)
12	Seed rate	140 kg/ha
13	Recommended fertilizer dose	80:40:40 (N:P:K)
14	Date of sowing	14 <sup>th</sup> December 2013
15	Date of harvesting	25 <sup>th</sup> March 2014



**Fig. 1:** Layout

### Details of irrigation scheduling

For the purpose of irrigation scheduling the irrigation in various treatments, predetermined soil moisture constants were used. Following equations were used for irrigation scheduling.

### Total available water (TAW)

Total available water is the amount of water that is available for plant use. It is actually the difference of soil moistures between field capacity and permanent wilting point. The total available water was calculated using following formulae;

$$TAW = \left[ \frac{\theta_{FC} - \theta_{PWP}}{100} \right] \times \gamma \times Z_r \times 1000 \quad \dots(1)$$

where,

TAW = Total available water, (mm).

$\theta_{FC}$  = Moisture content at field capacity, (%).

$\theta_{PWP}$  = Moisture content at Permanent wilting point, (%).

$\gamma$  = Bulk density, (gm/cm<sup>3</sup>).

$Z_r$  = Effective root zone depth, (m).

Using soil moisture constants, firstly total available water was determined for the experimental soil. For the purpose depth of effective root zone was taken 60 cm for wheat crop

**Depth of irrigation (IW):** After determining TAW, depth of irrigation was determined considering the maximum allowable depletion of 50 percent and using following equation 2.

$$IW = 0.50 \times TAW \quad \dots(2)$$

Where,

IW - Depth of irrigation to be applied in one irrigation, (mm).

**Cumulative pan evaporation (CPE):** Different moisture regimes were created by different irrigation schedules based on IW/CPE. For this purpose cumulative pan evaporation for respective treatments of IW/CPE ratios were determined using predetermined IW and values of ratios by using following equation 3.

$$CPE = \frac{IW}{Ratio} \quad \dots(3)$$



Pan evaporation data were recorded daily and cumulative figures were calculated subtracting the rainfall.

**Irrigation Scheduling in Control Treatment:** In control treatment, six irrigations were scheduled at six critical growth stages of wheat crop, viz. Crown Root Initiation (CRI), Maximum Tillering, Late Jointing, Flowering, Milking Stage and Dough Stage. In this treatment, depth of irrigation was determined by observing actual soil moisture before every irrigation and using following equation 4;

$$TAW = \left[ \frac{\theta_{FC} - \theta_{BI}}{100} \right] \times \gamma \times Z_r \times 1000 \quad \dots(4)$$

Where,

$IW$  = Irrigation water, (mm)

$\theta_{FC}$  = Moisture content at field capacity, (%)

$\theta_{BI}$  = Moisture content before irrigation, (%)

$\gamma$  = Bulk density, (gm/cm<sup>3</sup>)

$Z_r$  = Effective root zone depth, (m).

First common irrigation was given to all treatments just after sowing to bring the experimental plots to field capacity. For this purpose soil moisture content was determined before sowing to calculate the depth of irrigation of first common irrigation. In all plots water was conveyed through pipeline and measured quantity of water was applied using water meter.

### Yield of wheat

At the time of harvesting, net plot of size 5 m × 1.44 m was harvested from ground level along with earheads. Then it was weighted for getting biological yield per net plot. After threshing and winnowing the weight of grains were taken per net plot. To get straw yield, grain yield was subtracted from biological yield. After getting grain and straw yield per net plot, it was converted into yield per hectare.

## RESULTS AND DISCUSSION

The climatological data recorded at Meteorological Observatory, Department of Agronomy, Dr. PDKV, Akola during the period 14<sup>th</sup> December 2013 to 25<sup>th</sup> March 2014, were collected.

**Irrigation scheduling details:** Irrigation details were calculated and are given in Table 4.

**Table 4:** Irrigation scheduling details

Sl. No.	Particulars	Observation
1	Total available water (TAW), mm	149
2	Depth of irrigation (IW), mm	75
3	Cumulative Pan I <sub>1</sub> (IW/CPE=0.6)	125
	Evaporation at I <sub>2</sub> (IW/CPE=0.8)	93.8
	which irrigation I <sub>3</sub> (IW/CPE=1.0)	75
	scheduled treatment wise (CPE), mm I <sub>4</sub> (IW/CPE=1.2)	62.5

Thus, irrigation was scheduled at 125 mm CPE in treatment I<sub>1</sub> (IW/CPE = 0.6), at 93.8 mm CPE in treatment I<sub>2</sub> (IW/CPE = 0.8), at 75 mm CPE in treatment I<sub>3</sub> (IW/CPE = 1.0) and at 62.5 mm CPE in treatment I<sub>4</sub> (IW/CPE = 1.2). However in control treatment I<sub>5</sub>, irrigation was scheduled at 14, 28, 36, 57, 75 and 82 days after sowing at six critical growth stages of wheat.

### Amount of irrigation water applied to wheat

First common irrigation was applied in each treatment to bring the soil to field capacity. Depth of irrigation of this first common irrigation was determined on the basis of actual soil moisture content available before sowing. After sowing daily open pan evaporation observation was collected from Agro Meteorology Observatory of the university and cumulated previous evaporations. Irrigation was applied when predetermined cumulative pan evaporation reaches the respective value in different irrigation scheduling treatments of IW/CPE approach. However, in respect of control treatment the irrigation was scheduled at six critical growth stages of wheat.

It is seen from Table 5 that highest number of irrigations that is six irrigations were applied in treatment I<sub>4</sub> (IW/CPE = 1.2) and treatment I<sub>5</sub> (Control). Whereas, five irrigations were applied in treatment I<sub>3</sub> (IW/CPE = 1.0) followed by treatment I<sub>2</sub> (IW/CPE = 0.8) with four irrigations. However, in treatment I<sub>1</sub> (IW/CPE = 0.6), only three irrigations were applied. During the crop season 53.6 mm rainfall was received. It is observed that the total amount of irrigation water applied during crop season was found to be highest in treatment I<sub>4</sub> (541

**Table 5:** Irrigation scheduling details and Irrigation water applied

No. of Irrigation	I <sub>1</sub> (IW/CPE=0.6)		I <sub>2</sub> (IW/CPE=0.8)		I <sub>3</sub> (IW/CPE=1.0)		I <sub>4</sub> (IW/CPE=1.2)		I <sub>5</sub> (Control)	
	Date	IW applied (mm)	Date	IW applied (mm)	Date	IW applied (mm)	Date	IW applied (mm)	Date	IW applied (mm)
Common Irrigation after Sowing	14-12-2013	120	14-12-2013	120	14-12-2013	120	14-12-2013	120	14-12-2013	120
First	14-01-2014	75	08-01-2014	75	02-01-2014	75	30-12-2014	75	28-12-2013	69.66
Second	10-02-2014	74.6	28-01-2014	74.6	20-01-2014	74.6	14-01-2014	74.6	11-01-2014	79.01
Third	03-03-2014	46.4	14-02-2014	75	06-02-2014	75	29-01-2014	75	18-01-2014	57.91
Fourth	—	—	03-03-2014	46.4	18-02-2014	75	10-02-2014	75	10-02-2014	89.98
Fifth	—	—	—	—	03-03-2014	46.4	20-02-2014	75	27-02-2014	76.18
Sixth	—	—	—	—	—	—	03-03-2014	46.4	02-03-2014	47.91
Total		316		391		466		541		540.64

**Table 6:** Crop growth stage wise water requirement

Sl. No.	Crop growth stage	Water requirement under different irrigation scheduling				
		I <sub>1</sub>	I <sub>2</sub>	I <sub>3</sub>	I <sub>4</sub>	I <sub>5</sub>
1	After sowing	120	120	120	120	120
2	Crown root Initiation (14 DAS)	—	—	75	75	69.66
3	Maximum tillering (28 DAS)	75	75	—	75	79.01
4	Late Jointing (36 DAS)	—	75	75	75	57.91
5	Flowering (57 DAS)	75	75	75	75	89.98
6	Milking stage (75 DAS)	—	—	75	75	76.18
7	Dough stage (82 DAS)	75	75	75	75	47.91
	Seasonal water requirement	345	420	495	570	540.64

**Table 7:** Total water requirement of wheat

Treatment	Number of Irrigations	Irrigation water applied (mm)	Rainfall (mm)	Total water requirement (mm)	Saving water over control (%)
I <sub>1</sub> (IW/CPE=0.6)	3	316	53.6	369.6	38
I <sub>2</sub> (IW/CPE=0.8)	4	391	53.6	444.6	25
I <sub>3</sub> (IW/CPE=1.0)	5	466	53.6	519.6	13
I <sub>4</sub> (IW/CPE=1.2)	6	541	53.6	594.6	-0.06
I <sub>5</sub> (Control)	6	540.6	53.6	594.2	—

mm), followed by I<sub>5</sub> (540.64 mm), I<sub>3</sub> (466 mm), I<sub>2</sub> (391 mm) and I<sub>1</sub> (316 mm).

### Crop growth stage wise water requirement of wheat

Irrigation water applied during different growth stages and rainfall received during that stages summed up to determine the crop growth stage wise water requirement of wheat crop as influenced in different irrigation schedules and it is presented in Table 6.

It is seen from Table 6 shows that in case I<sub>3</sub> irrigation was not scheduled during maximum tillering. In

case of treatment I<sub>2</sub> irrigation was not scheduled during two growth stages i.e. crown root initiation and milking stage. Whereas, in treatment I<sub>1</sub> irrigations were not scheduled during three stages i.e. crown root initiation, Late jointing and milking stage.

### Total water requirement of wheat and saving of water

Total water requirement and saving of water as influenced by different treatments was presented in Table 7.

**Table 8:** Grain yield (q/ha) and straw yield (q/ha)

Treatments	Grain yield (q/ha)	Straw yield (q/ha)	Consumptive use in (ha-cm)	Water use efficiency (q/ha-cm)
I <sub>1</sub> (IW/CPE = 0.6)	19.05	59.42	34.46	0.55
I <sub>2</sub> (IW/CPE = 0.8)	26.71	55.24	41.96	0.64
I <sub>3</sub> (IW/CPE = 1.0)	30.95	85.72	49.46	0.63
I <sub>4</sub> (IW/CPE = 1.2)	32.50	94.59	56.96	0.57
I <sub>5</sub> – Control	27.37	78.88	56.92	0.48
Mean	27.32	74.77		
F' test	Sig.	Sig.		
SE(m)±	1.041	8.594		
CD at 5%	3.208	26.479		
CV (%)	7.623	22.988		

Total water requirement of wheat was found to be highest (594.6 mm) under irrigation scheduling at (I<sub>4</sub>), followed by I<sub>5</sub> (Control) (594.2), I<sub>3</sub> (IW/CPE=1.0) (519.6mm) and I<sub>2</sub> (IW/CPE=0.8) (444.6 mm). It was found to be lowest (369.6 mm) under irrigation scheduling at IW/CPE=0.6 (I<sub>1</sub>). Hence highest saving of water over control treatment was achieved in I<sub>1</sub> (38 %), followed by I<sub>2</sub> (25 %) and I<sub>3</sub> (13 %), whereas only (-0.06 %) saving of water was achieved in I<sub>4</sub> as compared to control treatment.

#### Yield (q/ha) and water use efficiency (q/ha-cm)

Grain yield and water use efficiency as influenced by different irrigation scheduling are presented in Table 8.

It is seen from Table 8 that treatment I<sub>4</sub> (IW/CPE=1.2) recorded highest grain yield and found to be at par with the treatment I<sub>3</sub> (IW/CPE=1.0). Grain yield recorded in treatment I<sub>5</sub> was at par with I<sub>2</sub>. Treatment I<sub>1</sub> (IW/CPE=0.6) recorded significantly lowest yield as compared to all other treatments. It is also seen that highest straw yield was recorded in treatment I<sub>4</sub> (IW/CPE=1.2), which was at par with treatment I<sub>3</sub> (IW/CPE=1.0) and treatment I<sub>5</sub> (control). Straw yield recorded at treatment I<sub>5</sub> were at par with I<sub>2</sub>. Treatment I<sub>2</sub> (IW/CPE=0.8) recorded lowest straw yield and found at par with treatment I<sub>1</sub>.

Highest grain and straw yield in I<sub>4</sub> and I<sub>3</sub> may be due to favorable soil moisture maintained in the root zone throughout the growing period of the crop. However soil moisture depleted below maximum allowable limit in case of treatments I<sub>1</sub> and I<sub>2</sub> resulted in stress and reduction in yields. Grain yield recorded in treatment I<sub>3</sub> (IW/CPE=1.0)

was significantly higher than that in treatment I<sub>5</sub> (Control) with saving of water of 13.11 %. Highest water use efficiency was recorded in treatment I<sub>2</sub>, which may be due to lower water use, followed by treatments I<sub>3</sub>, I<sub>4</sub>, I<sub>1</sub>, I<sub>5</sub> (Control). However, lowest WUE was recorded in treatment I<sub>5</sub>. Consumptive use in case of treatment of I<sub>1</sub> was lowest and whereas it was highest in case of treatment I<sub>4</sub>. It is also seen that water use in treatment I<sub>5</sub> was negligibly less than treatment I<sub>4</sub>, still water use efficiency in I<sub>4</sub> was more than I<sub>5</sub>. It may be due to higher grain yield recorded in treatment I<sub>4</sub> as compared to treatment I<sub>5</sub>.

#### CONCLUSION

Seasonal water requirement of wheat was found to be highest (570 mm) under irrigation scheduling at I<sub>4</sub>, followed by I<sub>5</sub> (Control) (540.64) IW/CPE = 1 (I<sub>3</sub>) (495 mm), I<sub>2</sub> (IW/CPE = 0.8) (420 mm) and I<sub>1</sub> (IW/CPE=0.6) (345 mm). It was found to be lowest (307.5 mm) under irrigation scheduling at IW/CPE = 0.6 (I<sub>1</sub>). Highest saving of water (38 %) over control treatment of six highest irrigations was achieved in I<sub>1</sub> (IW/CPE=0.6), followed by I<sub>2</sub> (25 %), I<sub>3</sub> (13 %) as compare to control treatment. Favorable soil moisture was maintained in the irrigation scheduling treatments of IW/CPE = 1.2 (I<sub>4</sub>) throughout the growing period and it was always maintained in allowable depletion regime. However, soil moisture was inadequate in irrigation scheduling at IW/CPE = 0.6 (I<sub>1</sub>). Whereas in irrigation scheduling treatments I<sub>2</sub> and I<sub>5</sub>, soil moistures were slightly depleted below allowable limit. Irrigation scheduling at IW/CPE = 1.2 (I<sub>4</sub>) recorded highest grain yield of wheat and found to be at par with treatments I<sub>3</sub> (IW/CPE = 1.0),





followed by  $I_5$  (Control–six irrigations) and  $I_2$  (IW/CPE = 0.8). Treatment  $I_1$  (IW/CPE = 0.6) recorded significantly lowest yield as compared to all other treatments. Grain yields recorded in treatments  $I_4$  and  $I_3$  were at par. Highest straw yield was recorded in treatment  $I_4$  (IW/CPE = 1.2), which was at par with treatment  $I_3$  (IW/CPE = 1.0). Lowest straw yield was recorded in treatment  $I_1$ . Highest water use efficiency was recorded in treatment  $I_2$  followed by  $I_3$ ,  $I_4$ ,  $I_1$  and  $I_5$ .

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# Induction of Seed Dormancy and its Impact on Seed Quality in Groundnut (*Arachis hypogaea* L.)

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## ABSTRACT

Field and laboratory experiments were conducted to induce dormancy in non-dormant groundnut variety GKVK-5 by foliar spraying of different dormancy inducing chemicals of various concentrations viz., maleic hydrazide @ 1250, 1500 ppm, abscisic acid @ 100, 200 ppm and cycocel @ 2000, 3000 ppm at 85 DAS during *kharif* 2020. The results revealed that there was a significant difference between the treatments of which the highest germination (82.00 %) was recorded from control while, the least germination (28.66 %) was recorded from maleic hydrazide @ 1500 ppm followed by (32.00 %) cycocel @ 3000 ppm immediately after harvest. Thus, the spray of dormancy inducing chemicals protect the seed from *in situ* germination due to rains at the time of harvest without affecting growth and yield parameters.

## HIGHLIGHTS

- Spraying of malic hydrozide @ 1500 ppm at 85 Days after sowing could induce dormancy in Groundnut cultivar GKVK-5 up to two weeks without affecting growth and yield parameters
- Spraying of dormancy inducing chemicals i.e MH (1250 and 1500 ppm) aba (100 and 200 ppm) Cycocel (2000 and 3000 ppm) could induce dormancy upto eight to twelve days in natural field conditions and upto 12 to 16 days in lab conditions after harvest
- The seeds treated with GA<sub>3</sub> @ 500 ppm recorded the highest germination (98.83%) for all the dormant seeds treated followed by hydropriming (98.00 %) and KNO<sub>3</sub> @ 0.6% (97.89%) in Groundnut cultivar

**Keywords:** Dormancy, Induction, Groundnut, Plant growth regulators

The groundnut (*Arachis hypogaea* L.) is one of the most important commercially grown oil seed crops in the world. It is also known as peanut, earthnut, manilla nut and monkey nut. In world, it is the 13<sup>th</sup> vital food crop and 4<sup>th</sup> most important oilseed crop. It is grown in nearly 26.40 million hectare worldwide with a total production of 37.10 million tonnes and an average yield of 14.00 q /ha while, India ranks first in groundnut area with 4.89 million hectares accounting for 17.32 per cent of the world area and second in production with 10.10 million tonnes accounting for 14.55 per cent of the world production and an average yield of 20.65 q/ha. In Karnataka, groundnut is grown in 0.57 million hectare with a production of 0.68 million tonnes and an average yield of 11.80 q/ha (Anon., 2020). Groundnut is widely known as peanut in many

countries though it is more a pea (a leguminous plant) than a nut. It is considered as a nut because of its high nutritional value. It is a less expensive and nourishing food. It contains 48-50 per cent oil and 26-28 per cent protein. It is a rich source of dietary fibre, minerals and vitamins such as biotin, copper, niacin, folate, manganese, vitamin E, thiamine, phosphorus and magnesium. It is primarily used as a vegetable cooking oil. It is also used in soapmaking, manufacturing of cosmetics and lubricants, olein stearin and their salts. Its oil is used as a substitute to oleic oil in pharmaceutical. It is harvested, processed

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and consumed as a snack food, peanut butter and as a candy. Groundnut kernels are consumed directly as raw, roasted, boiled or fried. Groundnut haulm is highly palatable (8-11% protein) fodder for cattle, when it is fed in green state. Its cake has massive value as feeding material for livestock and organic manure. It comprises 8 per cent N, 1.4 per cent P<sub>2</sub>O<sub>5</sub> and 1.2 per cent K<sub>2</sub>O. Globally, 50 per cent of the groundnut produced is used for oil extraction, 37 per cent for confectionery use and 12 per cent for seed purpose.

The cultivated groundnut *Arachis hypogaea* L. has two sub-species: sub species hypogaea (virginia bunch and virginia runner varieties) and sub species fastigiata (Spanish and Valencia varieties). The seeds of Spanish and Valencia bunch types are generally non-dormant or it naturally brakes more than a few weeks after seed maturity. Whereas, the seeds of virginia bunch and virginia runner varieties have an extended dormancy of four or more months (Rao, 1976). The non-dormant character in Spanish and Valencia bunch type is objectionable for groundnut cultivation as it causes heavy losses of produce by way of sprouting of pods in the field when the harvesting stage of crop coincides with the invariably received rain. The delayed harvest yields in germination of groundnut pods which are non-dormant to germination. This reduces not only the seed yield but also the quality significantly. It is an added problem in soils where water retention capacity is high. Due to *in situ* germination, a loss of 20 to 50 per cent in bunch groundnut pod yield has been reported (Nagarjun and Radder 1983).

The inability of certain seeds to germinate readily even when they are provided with all the conditions required for germination is known as seed dormancy. In general, in groundnut, bunch types are non-dormant while spreading and semi spreading types are having a varied period of dormancy.

Variety GKVK-5 is a high yielding variety with an average seed yield potential of 25 q / ha. It matures in 115 - 120 days with a shelling percentage of 75-80 and oil content of 49.6 per cent. The variety is moderately tolerant to major diseases like late leaf spot and rust and pests like thrips and leaf hopper. The main problem in this variety is lack of fresh seed dormancy, leading to *in situ* germination due to early onset of monsoon coinciding with harvest time.

## MATERIALS AND METHODS

The present investigation was conducted during *Kharif* 2020 at Agricultural Research Station, Balajigapade, Chickballapur. The experiment was conducted on sandy loam soil with 5.12 pH. The experiment consisted of seven treatments viz., T<sub>1</sub>-maleic hydrazide @ 1250 ppm, T<sub>2</sub>-maleic hydrazide @ 1500 ppm, T<sub>3</sub>-abscisic acid @ 100 ppm, T<sub>4</sub>- abscisic acid @ 200 ppm, T<sub>5</sub>-cycocel @ 2000 ppm, T<sub>6</sub>-cycocel @ 3000 ppm T<sub>7</sub>-control which were sprayed 85 DAS. The experiment was laid in a randomized block design with three replications. The gross plot size was 3.0 x 2.5 m. The row to row spacing was 30 cm, while plant to plant spacing was 15 cm. The fertilizers in the form of Urea, Di- ammonium Phosphate and Muriate of Potash were applied @ 25:160:60 kg/ha, respectively. First pre-sowing irrigation followed by second irrigation was given immediately after sowing. The crop was thoroughly weeded within the first 45 days. Once pegging began, soil disturbance was avoided. Usual agronomic practices of groundnut cultivation including those of its seed production were timely carried out during the growth period to raise a good crop.

### Pre-germinated pod (%)

The five tagged plants from each treatment were harvested and the number of pre-germinated pods were counted in each treatment. The pre-germinated pod percentage was worked out by using the following formula;

Pre-germinated pod (%) =

$$\frac{\text{No. of pre-germinated pods/plant}}{\text{Total number of pods per plant}} \times 100$$

### Germination (%)

The germination percentage was recorded at four days interval from the day after harvest. Initial germination was recorded on very first day after harvest. Three replicates of 50 seeds from each treatment were kept for germination at 25°C for 10 days using between paper (BP) method. The germination percentage was expressed on the basis of normal seedlings only as described in ISTA rules (Anon., 1999).

### Hundred Kernel weight (g)

One hundred kernels were randomly selected from each treatment and in three replications.

The weight of 100 kernels were recorded and expressed in grams (Anon., 1999).

### Shelling (%)

The observation on shelling percentage was taken at 15 days after harvesting. Two hundred fifty grams of cleaned and completely dried pods were taken from each treatment in three replications and shelled. Weight of the kernels was recorded and shelling percentage was worked out by using the following formula;

$$\text{Shelling (\%)} = \frac{\text{Weight of the kernels (g)}}{\text{Weight of pods(g)}} \times 100$$

### Sound mature kernel (%)

The observation on sound mature kernel per cent was taken at 65 DAH. The groundnut kernels were drawn randomly and weighed about 250 g in three replications in each treatment. The fully matured, uniform sized seeds were separated by discarding undersized, broken, immature and shrivelled seeds with the use of purity test board. The sound mature kernel percentage was worked out by using the following formula;

$$\text{SMK (\%)} = \frac{\text{Sound matured kernel (g)}}{\text{Total weight of kernel (g)}} \times 100$$

## RESULTS AND DISCUSSION

The results from the above experiment revealed that foliar application of dormancy inducing growth regulators did not record any significant difference between the treatments for any of the yield or its attributing parameters. Whereas, There was a significant difference for the pre-germinated pod percentage between the treatments. The highest germination (15.33 %) was recorded from control while the greatest induction of dormancy was influenced from the maleic hydrazide @ 1500 ppm which recorded mere 0.76 per cent germination followed by MH @ 1250 ppm (1.26 %). While, abscisic acid @ 100 ppm, abscisic acid @ 200 ppm and cycocel @ 2000 ppm showed on par results of pre germinated pod percentage. The results were on par with the Anand and Sharanappa *et al.* (2017).

This outcome could be ascribed to the decrease in the dampness content of the seed as it forestalls seed germination which shows that the use of maleic hydrazide kept the pods from engrossing dampness despite the fact that there was a lot of dampness in encompassing soil air. Barry and Peterson (1961) have additionally brought up the hindrance of growing in sweat potato because of decrease in the water ingestion limit of cells, as water assimilation is the essential stage in seed germination.

The various concentrations of dormancy inducing chemicals reduced the subsequent germination of seeds due to induction of dormancy as compared to control. The dormancy reduced as the time advanced in all the treatments. While, none of the dormancy inducing treatments could induce a complete

**Table 1:** Effect of plant growth regulators on yield and its attributing parameters in groundnut cv.

Treatment	Pods per plant	Graded pod yield (q/ha)	Shelling (%)	Days to maturity	100 kernel weights (g)	SMK (%)	Pre germinated pod (%)
T1	41.84	27.02	73.29	112.00	40.03	92.21	1.26
T2	38.26	27.65	74.77	115.67	40.84	94.90	0.76
T3	41.20	27.40	72.53	118.33	40.64	92.08	1.80
T4	38.99	26.92	75.28	116.00	39.32	93.94	1.70
T5	40.46	27.56	72.52	115.33	40.83	90.79	1.53
T6	39.52	28.41	72.94	120.67	40.03	94.56	1.30
T7	37.45	26.02	69.66	117.67	41.15	91.01	15.33
Mean	39.67	27.28	72.99	116.52	40.40	92.78	3.38
S. Em ±	1.38	0.54	1.08	1.85	1.08	1.36	0.25
C.D at 5%	—	—	—	—	—	—	0.78

**Table 2:** Influence of dormancy inducing treatments on germination percentage in groundnut cv. GKVK-5

Treatment	0 DAH	4 DAH	8 DAH	12 DAH	16 DAH
T <sub>1</sub>	34.00	42.66	47.33	66.00	77.33
T <sub>2</sub>	28.66	39.33	43.33	62.66	74.66
T <sub>3</sub>	41.33	52.66	63.33	73.33	83.33
T <sub>4</sub>	39.33	46.00	52.00	70.66	82.00
T <sub>5</sub>	36.00	46.66	57.33	70.00	76.66
T <sub>6</sub>	32.00	41.33	44.66	63.33	75.33
T <sub>7</sub>	82.00	86.66	91.33	94.00	96.66
Mean	41.90	50.75	57.04	71.42	80.85
S. Em ±	1.00	0.77	0.95	1.07	0.79
C.D at 5%	3.12	2.39	2.97	3.34	2.48

dormancy of up to 100 per cent. Spraying of maleic hydrazide @ 1500 ppm was the most effective in inducing dormancy (as the germination recorded 28.66, 39.33, 43.33, 62.66 and 74.66 per cent from the seeds that were put to germination on 0, 4, 8, 12 and 16 days after harvest) to a greater extent. It showed a reduction in germination of 53.34 per cent compared to control. It was followed by cycocel @ 3000 ppm which recorded 32.00, 41.33, 44.66, 63.33 and 75.33 per cent germination and it showed a reduction of 50 per cent in germination compared to control. On the otherhand, control recorded a germination percentage of 82 immediately after harvest eliciting no induction of dormancy.

The seed germination or dormancy primarily depends on the balance between water soluble growth promoters such as alpha amylase, catalase, auxin, ethylene, tryptophan, gibberellic acid etc, and growth inhibitors such as ABA and coumarin. Presence of auxin like substances is responsible for germination and making it non-dormant. The introduction of antiauxins to seeds by means of foliar spray at the time of kernel development suppresses the auxin formation and induce dormancy.

Dormancy or temporary suspension of germination is a complex phenomenon. Dormancy may block any of the sequential processes involved in germination. The work conducted earlier revealed that maleic hydrazide brings about certain physiological and biochemical changes, which ultimately make the seed dormant (Miracle *et al.*, 1955; Karivaratharaju and Rao, 1972 Ketring, 1977 and Bowley and Black, 1982).

The current examination obviously portrayed that maleic hydrazide is effective in diminishing the

ensuing seed germination as such by initiating seed dormancy for a time of around two weeks. As per one idea, the dormant condition of seed is reliant upon the general degrees of inhibitors and promoters present in the seed (Vaithilingam and Rao 1973). The non-dormant nature of bunch groundnut is because of the presence of development advancing water solvent auxin. The essential impact of maleic hydrazide on inciting dormancy appears to be through impedance in the tryptophan digestion, as the tryptophan is the forerunner in the blend of auxin (Nagarjun and Gopalakrishnan 1957 and Ketring 1977). Maleic hydrazide is found to build the substance of another amino acid, hydroxyl proline (Karivaratharaju and Rao 1972 and Vaithialingam and Rao 1973) which represses the auxin-initiated cell extension hence forestalling the seed germination (Cleland 1963).

The present study showed that if not the best at actuating dormancy, abscisic acid has extensively incited dormancy, the consequence of which was confirmed by Ketring (1973) and Narasimhareddy and Swamy (1979) by communicating the inhibitory activity of ABA on germination of groundnut seed. Sengupta and Sharma (1986) noticed the hindrance of groundnut seed germination by ABA. The inhibitory impact forced by ABA was turned around by kinetin application and they presumed that ABA and kinetin control the seed dormancy and germination, individually. ABA decreased the production of ethylene by preventing ethylene synthesis. It is an auxin antagonist. It inhibits the expression of alpha amylase gene. Its accumulation in the seedcoat strongly inhibits amylase activity. It inhibits cell division and cell elongation.

**Table 3:** Influence of dormancy inducing treatments on seedling vigour index-I (SVI-I) ingroundnutcv. GKVK-5

Treatments	Seedling vigour index-I				
	0DAH	4DAH	8DAH	12DAH	16DAH
T <sub>1</sub>	790	1000	1159	1776	2224
T <sub>2</sub>	669	990	1201	1836	2104
T <sub>3</sub>	999	1344	1766	1944	2398
T <sub>4</sub>	904	1068	1430	1970	2330
T <sub>5</sub>	856	1171	1493	1737	2199
T <sub>6</sub>	768	942	1160	1732	2128
T <sub>7</sub>	1941	2177	2452	2518	2787
Mean	990	1242	1523	1930	2310
SEm±	27.47	40.71	48.12	87.93	79.23
CD (P=0.05)	85.60	126.85	149.92	273.94	246.86

**Table 4:** Influence of dormancy inducing treatments on seedling vigour index-II (SVI-II) ingroundnut cv. GKVK-5

Treatments	Seedling vigour index-II				
	0DAH	4DAH	8DAH	12DAH	16DAH
T <sub>1</sub>	6464	8149	9430	13411	15821
T <sub>2</sub>	6105	7583	8799	10928	15575
T <sub>3</sub>	7709	10610	12525	14676	15932
T <sub>4</sub>	7722	9394	10449	13709	15109
T <sub>5</sub>	7298	8955	11726	14912	15064
T <sub>6</sub>	6015	8122	9210	11543	14680
T <sub>7</sub>	16698	17385	18703	20825	19449
Mean	8287	10028	11549	14286	15947
SEm±	344.30	502.52	547.05	598.51	713.81
CD (P=0.05)	1,072.60	1,565.59	1,704.29	1,864.62	2,223.85

Cycocel at both the concentrations has recorded a significant reduction of 46 and 50 per cent in the germination percentage as compared to control. Cycocel being a synthetic growth retardant inhibits seed germination by restricting the translocation of diffusible auxin from the plant apices to the germinating points. It prevents the production of gibberellic acid and inhibits the alpha amylase activity.

Seed germination is an energy requiring process and is therefore, dependent on the respiration of the seed, which in turn is dependent upon moisture content of the seed. Moreover, dormancy is also related to control of enzyme formation and inactivation of seed as catalase catalyst is known to assume a positive part during the time of seed germination. Further the decrease in the dampness content of the seed shows that the use of maleic hydrazide forestalls the pods from engrossing dampness

despite the fact that there is a lot of dampness in the encompassing soil climate during the period of pod advancement. Barry and Peterson (1961) have likewise pointed out the restraint of growing in sweet potato because of the decrease in water ingestion limit of cells, as water assimilation or hindrance is the essential stage in seed germination. The current discoveries are as per the consequences of Jayadeva (2008).

The non-dormant nature of bunch groundnut is because of the presence of development advancing water solvent auxin adversary, the essential impact of maleic hydrazide on initiating dormancy is by all accounts through obstruction in the tryptophan digestion, as the tryptophan is the antecedent in the amalgamation of auxin (Nagarjun and Gopalakrishnan 1957 and Ketring 1977). Notwithstanding this maleic hydrazide is found to expand the substance of another amino acid,



hydroxyl proline (Karivaratharaju and Rao 1972 and Vaithilingam and Rao 1973) which represses theauxin actuated cell lengthening (Cleland 1963).

The spray of dormancy inducing chemicals did not show any significant difference for the root length, shoot length, mean seedling length or mean seedling dry weight. However, it showed a significant difference in the seedling vigour index-I and seedling vigour index- II due to the significant difference in the germination percentage which was caused by the dormancy inducing chemicals sprayed at 85 DAS.

The seedling vigour index-I and II increased gradually with time in all the treatments including control due to the dissipation of dormancy over time. However, the highest to record the seedling vigour index I was from control which showed 1941, 2177, 2452, 2518 and 2787 on 0, 4, 8, 12 and 16 DAH respectively while the lowest to record was from maleic hydrazide @ 1500 ppm which showed 669, 990, 1201, 1836 and 2104 on 0, 4, 8, 12 and 16 DAH respectively.

The seedling vigour index – II recorded the highest value from control which showed 16698, 17385, 18703, 20825 and 19449 on 0, 4, 8, 12 and 16 DAH respectively while, the lowest value was recorded from maleic hydrazide @ 1500 ppm which showed 6105, 7583, 8799, 10928 and 15575 on 0, 4, 8, 12 and 16 DAH respectively. The various concentrations of dormancy inducing chemicals reduced the subsequent germination of seeds due to induction of dormancy as compared to control. The dormancy reduced as the time advanced in all the treatments. The current examination portrayed that maleic hydrazide is effective in diminishing the ensuing seed germination as such by initiating seed dormancy. The work conducted earlier revealed that maleic hydrazide brings about certain physiological and biochemical changes, which ultimately make the seed dormant (Miracle *et al.* 1955; Karivaratharaju and Rao 1972; Ketring, 1977 and Bowley and Black 1982). All the quality parameters are on par with the results of Jeevitha and Vasudevan *et al.* (2019).

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# Effect of different Moisture Content on the Physical Characteristics of Dill Seeds (*Anethum graveolens* L.)

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## ABSTRACT

Physical and chemical analysis of dill seed (*Anethum graveolens* L.) varies with variety, the region where it is grown and the stage of harvest. Determination of physical properties of seeds and agricultural products is important in the design of harvesting, handling, and processing equipment. Physical properties of dill seeds were determined at three levels of moisture content. All dimensions of seeds increased with increasing moisture content from 8.26% to 22.93%. The geometric mean diameter along with the coefficient of friction (0.81 to 0.93), angle of repose (32.85 to 42.56 degree), volume (3.03 to 3.58) and terminal velocity (2.29 to 2.87 m/s) increased with the different level of moisture content. Bulk density (432.81 to 412 kg/m<sup>3</sup>), true density (1132.6 to 1031.2 kgm<sup>3</sup>) and porosity (60.31 to 51.04 %) of the seeds exhibited a declining trend with an increase in moisture content while sphericity and surface area remained unchanged. The isolation of essential oil was conducted by hydro distillation with a yield of 1.42% per 50 gm of sample. Proximate analysis showed that dill seeds are good source of protein and dietary fibre.

## HIGHLIGHTS

- ① The article main focus is on how differing moisture contents affect the physical characteristics of dill seeds.
- ① The physical characteristics of dill seeds were significantly impacted by moisture content.
- ① With rising moisture content of dill seeds, the length, breadth, angle of repose, geometric mean diameter, and thermal velocity all increased.
- ① Moisture content increased with decreasing thickness, bulk density, actual density, porosity, and coefficient of friction of dill seeds.

**Keywords:** Dill seeds, different moisture content, physical properties

Dill, also known as *Anethumgraveolens*, is an annual fragrant plant from the Apiaceae (Umbelliferae) family that is native to the Mediterranean and West Asia. The common name dill comes from the Norse word *dylla* or *dilla*, which most likely means to calm, while the generic name *Anethum* is derived from the Greek word *anethon*. Dill is commonly known as dill (English), Soyaa (Unani), Sadakuppai (Siddha), Shatpushpaa (Ayurvedic), Sowa (Hindi), Sthatpushpi (Sanskrit) and Soya (Punjabi). The Indian dill plant usually known as sowa is cultivated throughout India, chiefly in Punjab, Uttar Pradesh, Gujarat, Maharashtra, Assam and West Bengal (Chahal *et al.* 2017).

The leaves of the annual plant dill are pinnately split. The plant may reach a height of 150 cm, with a circular stem and 2–5 branches that emerge from the stem's base and develop alongside the main stem. Yellow is the colour of flowers. The seeds develop a light brown hue and a fragrant odour as they mature. Broadly oval and compressed, the seeds are typically separate and free and measure

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4 mm long, 2-3 mm wide, and 1 mm thick (Pathak *et al.* 2014).

Jianu *et al.* (2012) extracted dill essential oil through steam distillation for 4 h and reported that the yield (% v/w) was 2.91% for mature seeds and 0.92% for immature seeds. Methanol, acetone, and hexane were used in a cold solvent technique by Mousavi *et al.* (2013) to generate dill extract. To ascertain the oxidative stability of sunflower seed oil, dill extracts were added at concentrations of 0.5, 0.3, 0.1, and 0.05% w/w. By measuring the peroxide value and the induction duration using the rancimat instrument and the samples that included extracts, the antioxidant activities of the samples were assessed. They discovered that the sample with the highest oxidative induction duration and the lowest peroxide value included 0.5% methanolic extract of dill. Limonene (14.61%), carvone (62.48%) and dillapiole (19.51%), and limonene are all present in dill seed essential oil, according to Said-Al Ahl *et al.* (2016).

It is essential to understand the physical characteristics of dill that are affected by moisture, such as its longitudinal dimensions, porosity, true density, and bulk density, in order to design processing machinery, techniques, and storage facilities that work well. The design of efficient oil extraction, dehulling, and hull separation machinery depends on the food commodities' aerodynamic and storage structural qualities.

The goal of the current study is to identify moisture-dependent physical characteristics of dill seed at a moisture range of 12.3% dry basis (d.b.), including spatial dimensions, geometric mean diameter, sphericity, surface area, volume, 1000-seed mass, bulk density, true density, porosity, terminal velocity, static coefficient of friction and angle of repose.

## MATERIALS AND METHODS

All measurements of different moisture content on physical properties were performed in triplicate. The values of the various parameters were articulated as the mean  $\pm$  standard deviation (SD).

### Plant material

Fresh, mature and good quality mature dill seed of cultivar Gujarat Dill seed-2 were procured from Mehsana Beej Nigam, Mehsana, Gujarat. All

contaminants, including as dust, chaff, stones, and broken seeds, were removed from the seeds during cleaning.

### Determination of Physical Properties

The geometric mean diameter and sphericity of each seed were determined using the following equation. (Mohsenin 1986).

$$\text{Geometric Mean Diameter, } D_g = (L * B * T)^{\frac{1}{3}} \quad \dots(1)$$

$$\text{Sphericity, } (\phi) = \frac{(L * B * T)^{\frac{1}{3}}}{L} \quad \dots(2)$$

Where,

$L$  = Longest intercept, (Length) in mm;

$B$  = Longest intercept normal to 'L' (Breadth) in mm;

$T$  = Longest intercept normal to 'L' and 'B' (Thickness) in mm.

The surface area of the seed was determined using the following equation (Altuntas *et al.* 2005);

$$\text{Surface area, } (S_a) = \Pi * (D_g)^2 \quad \dots(3)$$

Where,

$D_g$  = Geometric mean diameter, (mm)

### Bulk density

Bulk density ( $\rho_b$ ) was determined by filling a 500 ml cylinder with dill seeds at a constant rate without compressing and then weighing the contents of the cylinder. Bulk density was calculated by using the equation mentioned below;

$$\rho_b = \frac{M_x}{V_c} \quad \dots(4)$$

Where,

$\rho_b$  = Bulk density, (kg/m<sup>3</sup>)

$M_x$  = Weight of sample, (kg)

$V_c$  = Volume of the container, (m<sup>3</sup>)

### Angle of repose

A vertical cylinder formed of a sheet that was open at both sides and filled with ajwain seed before being gently raised to measure the angle of repose of the seed was used (Dutta *et al.* 1988). The common Equation 5 was used to get the angle of repose.

$$\text{Angle of repose, } \theta = \tan^{-1} \frac{2h}{d} \quad \dots(5)$$

Where,

$h$  = height of the cone (mm)

$d$  = diameter of the cone (mm)

### Coefficient of friction

The static coefficient of friction for the dill seeds was determined on galvanized steel. A topless and bottomless box was used to determine the coefficient of static friction the surface was raised gradually until the filled cube just start to slide down, the angle at this point was recorded and the coefficient of static friction was calculated using the formula given below (Orhevba *et al.* 2013).

$$\mu = \tan \theta \quad \dots(6)$$

### True density

True density ( $\rho_t$ ) was determined by using the toluene displacement method as toluene is less absorbed by the seeds when compared to that of the water. A known quantity of seeds was immersed in toluene and the volume displaced by toluene was

recorded. True density was calculated by using the equation mention;

$$\rho_t = \frac{M_x}{V_c} \quad \dots(7)$$

Where

$\rho_t$  = True density (kg/m<sup>3</sup>)

$M_x$  = Weight of sample (kg)

$V_c$  = Volume of toluene displaced in the cylinder (m<sup>3</sup>)

## RESULTS AND DISCUSSION

Table 1 shows the axial dimension,geometric and arithmetic mean diameter, sphericity, surface area, bulk and true density, porosity, volume, coefficient of friction, angle of repose, and terminal velocity of dill seed at different moisture content 8.26%, 15.03% and 22.96% (d.b.). This result indicates that the percent increase in geometrical parameters with moisture content is reported in Table 1. It showed that drying of dill seed at 8.26%, 15.03% and 22.96% (d.b.) Shrinkage occurs as a result of a reduction in seed dimensions caused by moisture content. Zewdu (2011) and Gharib-Zahedi *et al.* (2011) both observed an increase in seed dimensions for ajwain and black cumin (2010).

**Table 1:** Effect of moisture content on physical properties of dill seeds

Seed dimensions	Moisture content					
	8.26%		15.03%		22.96%	
	Mean	SD	Mean	SD	Mean	SD
Length (mm)	3.99	0.0942	4.03	0.095	4.27	0.096
Width (mm)	1.84	0.044	1.916	0.0456	2.09	0.05
Thickness (mm)	1.19	0.0285	1.273	0.0304	1.31	0.0313
Bulk density (kg/m <sup>3</sup> )	432.81	10.35	420.536	10.059	412.58	9.869
True density (kg/m <sup>3</sup> )	1132.6	27.093	1114.687	26.664	1031.2	24.667
Angle of repose	32.85	0.786	37.81	0.904	42.56	1.018
Porosity (%)	60.31	1.442	54.943	1.314	51.04	1.221
Coefficient of friction	0.81	0.0562	0.873	0.0547	0.93	0.0603
Arithmetic mean diameter (mm)	2.35	0.562	2.243	0.0536	2.52	0.0602
Geometric mean diameter (mm)	2.08	0.0497	2.237	0.0535	2.24	0.0536
Sphericity	0.54	0.0129	0.54	0.0129	0.54	0.0129
Surface area	0.87	0.0208	0.8	0.0191	0.87	0.0208
Terminal velocity (m/s)	2.29	0.0558	2.433	0.0582	2.87	0.0686
Volume	3.03	0.0725	3.497	0.0347	3.58	0.0866



The geometric mean diameter of the seed was discovered to be greater than its breadth and thickness. Sphericity did not alter considerably when moisture content grew, although surface area did (Table 1). Previous study has shown that moisture content may impact sphericity in a variety of ways. Baryesh and mangope (2002) found that when moisture content increased, pigeon pea sphericity decreased. However, Zewdu (2011), Sobukola and Onwuka (2011) observed that an increase in moisture content was associated with an increase in the sphericity of ajwain and locust bean seeds. Gharib-Zahedi *et al.* (2010) and Isik and Unal (2007) showed a linear rise in terminal velocity with moisture content of black cumin and white speckled kidney beans. As moisture increases, terminal velocity likewise increases. Dill seed angle of repose rose from 32.52 to 44.05° for seed in the moisture range of 8.26% to 22.96% at various moisture levels (d.b.)

## CONCLUSION

While sphericity was unaffected by a rise in moisture content, the dimensions of dill seed rose linearly with moisture content, including length, breadth, thickness, geometric mean diameter, volume, and surface area. With an increase in moisture content from 8.26% to 22.96%, bed porosity, bulk density, and true density all dropped linearly from 60.31 to 51.04%, 432.81 to 412.58 kg/m<sup>3</sup>, and 1132.6 to 1031.2 kg/m<sup>3</sup>, respectively (d.b.). In the studied moisture range, the angle of repose and terminal velocity rose from 32.85° to 42.56° and 2.29 to 2.77 m/s, respectively.

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# Development and Characterization of Environment Friendly Starch and Protein Based Packaging Materials for Food Applications

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## ABSTRACT

The non-biodegradability of synthetic polymer based packaging materials leads to environmental concern to the society, opened the way for eco-friendly biodegradable materials which come from agro-food industry wastes which will be helpful for food products packaging. Different preliminary trials were conducted with broader range of different ingredients and plasticizers to study the detailed overview of these variables. Corn starch, whey protein concentrate, whey protein isolate, carrageenan, polyvinyl alcohol (PVA) taken as base component for the formation of film. Glycerol 2% was used as plasticizer in composite film applications because of its plasticization ability due to its low molecular weights and give better handling properties like flexibility and elasticity. From the studies, it was concluded that the addition of corn starch @ 2.5%, whey protein concentrate and whey protein isolate each @ 2.5%, carrageenan @ 0.25%, poly vinyl alcohol @ 0.5% having better film forming properties and easy to peel off for application in food products. The developed film also having better biodegradable in nature which will be helpful to reduce to environmental burden.

## HIGHLIGHTS

- ① Corn starch, Whey protein concentrate, Whey protein isolate, Carrageenan, Polyvinyl alcohol (PVA), Glycerol taken as components for the formation of film.
- ② Having better film forming and biodegradable properties proven to be environmental friendly packaging materials helpful for food products applications.

**Keywords:** Environment, Starch, Protein, Packaging materials, Food application, Film Properties

Conventionally, polyethylene is used for packaging of food products which is a synthetic polymer. Food packaging, mainly deals with preservation and protection of all types of foods from sensory, physico-chemical and microbial spoilage (Wróblewska *et al.* 2018). Biodegradable polymeric compounds are an alternative to synthetic polymers for food packaging, as they are abundant and renewable (Cazón *et al.* 2017). Unfortunately, due to the poor barrier properties and weak mechanical properties demonstrated by natural polymers, the use of biodegradable films for food packaging has been

strongly limited. Whey based films can be obtained from whey protein concentrates (WPC) and isolates (WPI). Whey protein concentrate and whey protein isolate, are biopolymers that has received much attention for use as a potential edible or biodegradable food packaging material because these has been shown to produce transparent films

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and coatings that can act as an excellent oxygen barrier property (Sothornvit & Krochta 2005). Starch has been evaluated in its film-forming ability for applications in the food packaging area (Jiménez *et al.* 2012). Carrageenan has shown good filmogenic properties (Han & Kim 2008). PVA is a synthetic, water soluble polymer with excellent film forming, good tensile strength, emulsifying, biodegradability and adhesive properties (Kanatt *et al.* 2012). Keeping in view of the above points, the present study was carried out with to optimize the above mentioned ingredients and develop the composite film by partial substitution of synthetic polymers (PVA) with natural polymers with the aim to develop environment friendly packaging materials for food applications.

## MATERIALS AND METHODS

### Development of Environmental Friendly Starch and Protein Based Packaging Materials

Composite films were developed from a variety of sources, such as corn starch, whey protein concentrate, whey protein isolate, carrageenan with the addition of plasticizer as a glycerol. The film prepared by wet method was easy, included by dispersion or gelatinization, homogenization, casting and drying. The plasticizer (glycerol) was tried at two different levels i.e. 2 and 3 per cent and the concentration of corn starch varied from 2.5-6 per cent. The concentration of WPC & WPI also varied from 2.5-6 per cent. The poly vinyl alcohol (PVA) varied between 0.5% to 1%. Different combinations were tried for the development of composite film. Required quantities of poly vinyl alcohol, whey protein concentrate, whey protein isolate and carrageenan was added with water for proper mixing of ingredients. After that addition of corn starch was added then heating (68 °C) continued upto gelatinization. Gelatinization was occurred depending upon type and amount of corn starch and glycerol was used. Casting container such as plastic petri dishes (150 mm) was used for the formation of film, and then it was transferred to hot air oven at 40°C for 16-17 hours for drying of the film.

### Characterization of film

Film thickness was measured with the help of

digital vernier caliper, six measurements were taken at different points randomly selected from each film and an average was obtained. UV-VIS spectrophotometer was used to find out the light transmittance of the film at the wavelength of 660 nm. Water activity meter was calibrated with water at 25°C and then used to measure the water activity of the film. The water solubility of the film was measured by the procedure followed by Singh *et al.* (2015). Water vapor permeability was measured using a modified method (ASTM, 2000).

**Mechanical properties of film:** Tearing strength and Puncturing strength was measured by TMS- Pro Food Technology Corporation texture analyser. The minimum force required to rupture and pierced the film was calculated in N (Newton). Each test film requires at least six replicate measurements.

**Instrumental colour measurement:** The instrument CR-400 chroma meter was calibrated as specified by the manufacturer of the equipment. Data was received through the software in terms of L\* (lightness), ranging from 0 (black) to 100 (white), a\* (redness), ranging from +60 (red) to -60 (green) and b\* (yellowness), ranging from +60 (yellow) to -60 (blue) helpful to find out the colour values of the film.

The biodegradation of the film was measured by the procedure followed by Al-Sahlany 2017.

## RESULTS AND DISCUSSION

Different preliminary trials were conducted with broader range of different ingredients and plasticizers to study these variables for formation of film. Based upon the preliminary trails the composite with PVA film was prepared with the addition of corn starch @ 2.5%, whey protein concentrate and whey protein isolate each @ 2.5%, carrageenan @ 0.25%, poly vinyl alcohol @ 0.5% resulted with very good acceptability and better film forming properties as compared to other combinations. This film was easy to peel off without any fear of getting torned off. Addition of corn starch leads to gelatinization of corn starch, this can be done by using excess water, i.e. granules get disrupted in excess water. Then glycerol was added and mixed properly with the help of magnetic stirrer to increase the film solubility, lightness, water absorption and produce more compact structures. Glycerol reduces the degree of crystallinity. The

developed film was shown in Fig. 1.



**Fig. 1:** Optimized film consists of CornStarch, Whey protein Concentrate, Whey protein Isolate, Polyvinyl Alcohol, Carrageenan and Glycerol

### Quality characterization of film properties

The film properties were shown in Table 1. The film thickness of composite with PVA was 0.091 mm. The results correlated with the findings of Rhim & Wang (2013). Due to addition of PVA to the films, the thickness increases which resulted in decrease of light transmittance. The transmittance of the developed film was 81.28%. The same was reported by Yang *et al.* (2018). The water activity of the film was 0.3899. The addition of PVA, filling the pores in the macromolecules structures which decreases permeability to water vapour and occupy the sites on composite films that normally would be occupied by water. Due to this reason, the WVP value was 6.09(g/m<sup>2</sup>h). Film Solubility value is an important functional property for the film based on biopolymers, which is related to the hydrophilicity of the materials. Here, the value was 29.76%.

The incorporation of PVA along with other ingredients into the film solutions effectively enhanced the interfacial interactions within the film network via the establishment of hydrogen and covalent bonds between polymer molecules. The tensile strength and puncturing strength was 9.36(N), 3.12(N) respectively. The findings correlated with Wu *et al.* (2009).

The objective of methods of evaluation of colour of films provides us quantitative view on the effect of film on product appeal, consumer acceptability and

market ability. L\*, a\* and b\* value of tested samples is shown in Table 1. The L, a and b values were 36.42,-0.89 and -2.20 respectively.

**Table 1:** Properties of the Optimized Film (n=3)

Parameters	Values
Film Thickness (mm)	0.091
Light transmittance (%)	81.28
Water activity (Aw)	0.3899
Water vapour permeability (g/m <sup>2</sup> h)	6.09
Film Solubility (%)	29.76
Tensile Strength (N)	9.36
Puncturing Strength (N)	3.12
Colour value of the film	L = 36.42 a = 0.89 b = -2.20
Biodegradability (50days) %	92.33

The Biodegradability of composite with PVA was 92.33%. Biodegradation involves enzymatic and chemical degradation by living organisms. The films which has more water absorption capacity will degrade fast. Similar results were also observed by the Azahari *et al.* (2011).

### CONCLUSION

From the research findings, it was concluded that incorporation of above mentioed quantities of polyvinyl alcohol, corn starch, whey protein concentrate, whey protein isolate, carrageenan and plasticizer glycerol resulted with better film forming properties along with biodegradability for usage as environmental friendly packaging materials for food applications.

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# Artificial Intelligence Based Grading System for Mango Fruit- A Review

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## ABSTRACT

Mango is major fruit crop in India which accounts 40% share in total fruit export. In mango-processing industries and during export of mango accurate grading and classification is an essential post-harvest unit operation. However, these processes are carried out manually which are tedious and leads to some errors and low accuracy that directly effects its quality. Thus, substituting the traditional labor-intensive technique by automated technologies based on artificial intelligence will not only increase operation efficiency but also optimize accuracy, labor saving, and appropriate handling of fruits. Also, premium quality fruits can be sorted separately for export. The main content of this article is to study methodology which utilizes digital fuzzy image processing for grading mangoes and development of effective algorithm for segregating mangoes with greater accuracy as compared to manually grading. Also, this study focusses on computer vision-based grading technique which uses image processing and extraction of feature based on grading parameter by combining artificial intelligence and many more grading systems based on different parameter. This study will help farmers to evaluate mango quality before export and help to procure profitable income.

## HIGHLIGHTS

- ① Substitute for traditional labor-intensive technique by automated technologies based on artificial intelligence.
- ① Fuzzy image algorithm for segregating mangoes with greater accuracy.
- ① Computer vision-based grading technique which uses image processing and extraction of feature based on grading parameter.
- ① The grading accuracy noticed in fuzzy image analysis was 80%.
- ① In computer vision-based grading the grading accuracy is more in terms of colour grading the accuracy rate is 95%, accuracy rate for surface defect is over 90% and mango size error is near about 3%.

**Keywords:** Artificial Intelligence, Grading, Mango, Image Analysis

India is land of agriculture and also leading producer and exporter of various fruits, pulses, spices and many more. Thus, before export of agricultural product it has to go through some processes which determine whether it is of export quality or sold in domestic market or rejected. In India, all these processes mainly sorting, grading, classification etc. are carried out manually. These techniques are based on traditional methods

which are inaccurate, time consuming and prone to errors and it does not satisfy market demand in terms of quality. Also, the shortage of farm labors and increasing labor cost are major problems faced during the process. On the other side use of

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automated systems not only increases productivity but also increase speed, high accuracy, reduces labor cost and proper handling of fruits.

Mango (*Mangifera Indica* L.) is a tropical fruit tree and is known as 'King of fruits. Variety of mango are classified according to its characteristic and origin. The major varieties of mango found in India are Alphonso-Ratnagiri Maharashtra, Kesar-Junagadh Gujrat, Dasher-Lucknow Uttar Pradesh, Badami-Karnataka. Presently production of mangoes has been increased considerably through improved varieties to meet customer demand and preference (Pitas, 2000). Mango grading standards plays an important role in classifying mangoes. Color and size of fruit are used for sorting (Ganiron, 2014). Proportion of factor is most important factor for determining mango quality. The proportion of mangoes is identified by maturity or age of mango. According to international standard the proportion of mangoes ranging from 1.0 to 1.1 is best quality mango (Thin *et al.* 2020). Presently classification of mangoes is based on physical characteristic. It is done manually and is greatly dependent on labors knowledge and experience. Appropriate and accurate classification is very important for export (Ganiron 2014).

Digital image processing analysis and computer vision plays a leading role in field of telecommunication, broadcasting medical imaging, multimedia system and intelligent sensing system, remote sensing and printing (Pitas 2000). Companies are moving towards automated grading. One of researcher has made apple grading system based on image analysis. The system was trained in such a way that it was feed with different samples to make it expert in creating data (Blasco *et al.* 2003). During grading process of mango, its characteristic is taken into consideration. Identification of size is done by machine vision by measuring area and dimension of fruit. For stem location random Images of different fruits are taken.

Now-a-days Computer vision system is used in agricultural grading application. Computer vision-based system is used in field of crop monitoring, precision farming, process automation, aerial surveillance, robotics, quality control, nondestructive inspection of product properties, remote sensing and many other fields. In computer vision system grading is based on maturity, size

and surface defects (Nandi *et al.* 2014). computer vision is based on information from image captured from the real time. Computer vision is duplicate of human vision with higher accuracy. Also computer vision is highly effective tool with fast and non-contact quality and grading evaluation tool has become boon for food processing industries. Computer vision systems have been developed for agricultural grading applications such as direct colour mapping system to evaluate the quality of tomatoes and dates (Lee *et al.* 2010), automated inspection of golden delicious apples using colour computer vision (Varghese 1991), intelligent apples quality estimator using machine vision (Chauhan *et al.* 2012), automated strawberry grading system based on image processing (Liming *et al.* 2010) and colour vision system for peach grading (Miller *et al.* 1989), sorting of sweet Tamarind (Jarimopas *et al.* 2008). Development of *Jatropha Cruces* colour grading system for ripeness evaluation using RGB colour space because of its basic synthesis property and direct application in the image display (Effendi *et al.* 2009).

The study of these system is useful for controlling and upgrading the quality of mango fruit for exporting purpose. More significantly the use of grading methods based on artificial intelligence will enhance quality, identify pattern in order to upgrade automation in agriculture production in India.

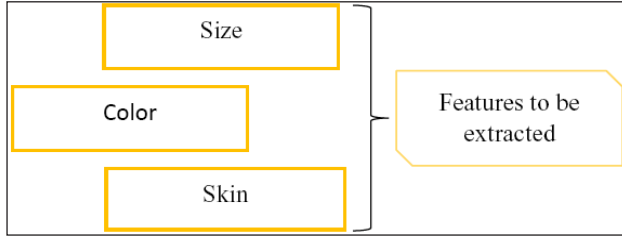
The main objective of this study is to provide an overview on various parameters and methods used for gradation of mango. This paper consists of method that incorporates fuzzy sets and approximate reasoning in fuzzy image processing to determine the quality grade of mangoes. It includes the study of computer vision-based system based on maturity level, size and surface defects. The organization of paper is done as follows 'Mango grading by fuzzy image analysis' is discussed in section 1 and Mango grading by computer vision is discussed in section 2.

## MATERIALS AND METHODS

### 1. Mango Grading Method by Fuzzy Image Analysis

The grading through fuzzy image analysis is done on basis of size, color and skin. This fuzzy image analysis can be applied in mango grading in MATLAB by collaborating the digital image

processing and fuzzy classification.

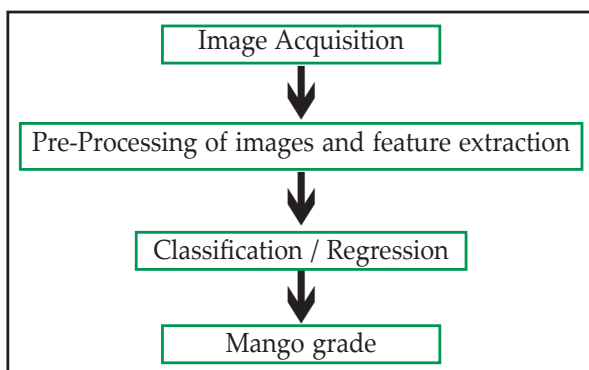


This study proposes a mango grading method for mangoes quality classification by using fuzzy image analysis.

The Algorithm of Proposed Method Consisted of Following Steps: (Razak *et al.* 2012):

- ♦ **Step 1:** Determine the size of mango by calculating the area of mango.  
The size is most common feature and is determined using machine vision by measuring the areas or diameter of mango.
- ♦ **Step 2:** Detect the color of mango by determine the mean of Three-color array for red, green and blue.  
$$\text{Mean image} = (\text{Red value (Find size image)} + \text{Green value (Find size image)} + \text{Blue value (Find size image)})/3$$
- ♦ **Step 3:** Apply edge detection algorithm to determine skin of Image mango  
$$\text{Mean skin} = (\text{edge (Red value)} + \text{edge (Green value)} + \text{edge (blue value)})/3$$
- ♦ **Step 4:** Fuzzy Inference Rule is applied for three values of Size, color and skin to compute the grade of mango.
- ♦ **Step 5:** Rank the mango quality based on mango grade.  
The mangoes are ranked on basis of Grade A, Grade B, Grade C categories.

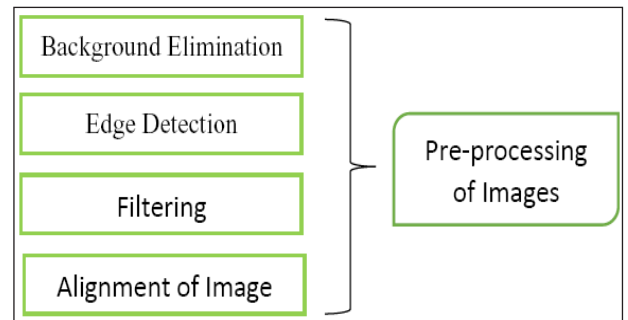
## 2. Mango Grading by Computer Vision



### Step 1: Image Acquisition

Computer vision system is majorly depended on quality of image. Colored images are taken with help of cameras at different angles using white background (Ganiron 2014). Special setup for multiple view acquisition is needed (Vélez-Rivera *et al.* 2014). Its video acquisition can be done while mango passes through conveyer belt (Nandi *et al.* 2016). Each image consists if single mango (Supekar *et al.* 2020).

### Step 2: Pre-Processing of Images and Feature Extraction



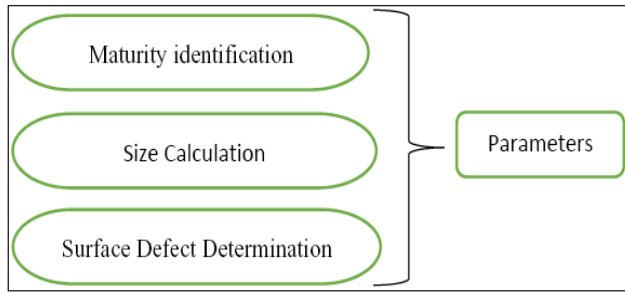
Operations such as noise removal, image enhancement, histogram equalization, contrast sharpening, morphological operations, image smoothening, brightness improvement, shadow removal etc. are applied (Bermúdez *et al.* 2017). Image pre-processing is performed in MATLAB or Python. In order to upgrade and perform proper image analysis and feature extraction, many image pre-processing after the image acquisition the image obtained is with background. Thus, to extract the characteristic feature of mango the background should be eliminated. Along with background elimination edge detection, filtering, alignment of image is also performed.

### Features Extraction

After background elimination the features required for grading parameters are extracted from image. These extracted features help in distinguishing mangoes. Colour features are extracted for ripeness analysis (Vélez-Rivera *et al.* 2014) (Pandey *et al.* 2014) (Limsripraphan *et al.* 2019). Geometric properties like area, perimeter, major axis, minor axis is computed for size estimation (Pandey *et al.* 2014). Shape related properties like eccentricity, cross-ratio (Naik *et al.* 2015), Fourier descriptors (Nandi *et al.*



2016), are obtained for shape analysis. Defect area is computed for defect-based analysis (Ganiron 2014) (Vélez-Rivera *et al.* 2014) (Nandi *et al.* 2016).



### Maturity Identification

Maturity Identification of mango can be estimated by using support vector machine (SVM) based classifier for extracted features (Nandi *et al.* 2014). For this mango image and binary mango image of different angles and positions is used.

### Size Calculation

This is one of important attribute for grading because in local market as well as foreign market bigger size of mangoes are preferred by costumer. Thus, this can be determined by maximum major axis and maximum minor axis of mango image. For this the mango image should be separated from its background (Nandi *et al.* 2014). The length, breadth and thickness of mango fruit is measured by vernier’s scale.

### Surface Defect Determination

Surface defect includes the black spot, scratches while harvesting etc. these factors also affects the quality of fruits. These defects are very critical and tough to identify. The images of mangoes are taken by digital camera and percentage of surface area defect is calculated.

### Step 3: Classification / Regression

Different machine learning classifiers like support vector machines (Agilandeewari *et al.* 2007) (Limsripraphan *et al.* 2019), decision trees, naïve bayes (Bermúdez *et al.* 2017) (Limsripraphan *et al.* 2019), neural networks (Alejandro *et al.* 2017) (Yossy *et al.* 2017), fuzzy classification (Pandey *et al.* 2014) (Naik, *et al.* 2015), k-nearest neighbour (Ganiron 2014) etc can be employed for mango classification. Parameter specific classification can

predict mangoes as (unripe/half ripe/overripe), (small/medium/large), (deformed/well-formed) etc. Regression can be used to predict the mango shelf-life (Nandi *et al.* 2016), mango length, breadth etc.

### Step 4: Mango grade

Classification of mangoes is according to standard and graded as (Grade A, Grade B, Grade C, Grade D). On the basis of these grades, it is decided that whether the mango is of export quality, domestic quality or reject.

## RESULTS

This study summarizes use of fuzzy image analysis for grading mango in MATLAB to extract the parameters like size, color and skin by using effective algorithm. Secondly, the computer vision-based mango grading by using various pre-processing methods. We also studied grading by fuzzy image analysis has grading accuracy about 80% and in computer vision-based method grading accuracy is more in terms of colour grading which is about 95%, accuracy rate for surface defect is over 90% and mango size error is near about 3%.

## CONCLUSION

In this paper different methods for classification used for grading for mango fruit based on various parameters are reviewed. External features like mango size, shape, ripeness and defect can be extracted from mango image using image processing techniques.

It has been observed that this method was carried out in MATLAB language and it is useful for different fuzzy environment. The advantages of this system are that its interference engine does not depend on human experts. This system with appropriate reasoning makes the best grading as compared to human expert.

This system is substitute to human expert. The proposed a model to grade mangoes which include hardware and software. Therefore, it has been concluded that computer vision-based model can be successfully used to classify mangoes into different grades.



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# Studies on Effect of Organic Manures and Seed Priming on Growth and Seed Yield in Paddy (*Oryza sativa* L.)

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## ABSTRACT

A field study was conducted at NSP, UAS, GKVK, Bengaluru during *kharif* 2019 to study the effect of organic manures and seed priming on seed yield and quality in paddy. Experiment comprised of 12 treatment combinations involving three priming treatments and four levels of fertilizer sources laid out in factorial RCBD design with three replications. Among different treatment combinations priming with 20 % *Pseudomonas fluorescense* and application of RDF (Urea-217.4 kg + SSP-312.5 Kg + MOP-83.33 kg/ha) recorded maximum plant height, at harvest (80.87 cm), maximum number of tillers per hill (16.66), number of panicles (15.89) and panicle length (20.24 cm). Application of organics (125 kg neem cake + 10 tonnes FYM + 10 kg PSB per ha) was the next best treatment (79.07 cm, 16.20, 15.10 and 19.64 cm respectively). Seed yield parameters viz., number of filled seeds per panicle, seed yield per plant and seed recovery recorded by priming with 20 % *Pseudomonas fluorescense* and application of organics (112.93, 19.05 g and 94.29 respectively) are on par with the RDF treatment (114.09, 19.43 g and 97.74 respectively).

## HIGHLIGHTS

- ① RDF has recorded the highest yield parameters [number of tillers (18.17), panicle length (19.73 cm) and seed yield (48.30 q/ha)] followed by treatment N<sub>3</sub> i.e., FYM + PSB + neem cake (17.32, 18.60 cm, 45.40 q/ha, respectively)
- ② Quality parameters are recorded highest by the application of FYM + PSB + neem cake [test weight (15.26 g), germination (90.71%), mean seedling length (17.71 cm), seedling dry weight (107.31 mg), SV-I (1630) and SV-II (9749)].

**Keywords:** *Kharif*, neem cake, seed, paddy, *Pseudomonas fluorescense*, treatment

Paddy (*Oryza sativa* L.) is the major staple food for more than half of the global population it is considered as the “global grain”. Asian countries consume about 90 per cent of the rice grown and produced in the world. Paddy belongs to grass family *Poaceae*. It is said to have two important cultivating species *Oryza sativa* L. (Asian rice) and *Oryza glaberrima* L. (African rice). *Oryza sativa* L. have 3 subspecies Japonica, Javanica and Indica.

Modern intensive agriculture is heavily dependent upon the chemical fertilizers for meeting the nutrient demand in paddy. The chemical residues of fertilizers and pesticides accumulated in the soil year after year have rendered our soil lifeless with

its structure hardening. Hence, organic agriculture i.e., the use of available organic resources such as FYM, compost, green manure, green leaf manure, sewage sludge, urban waste, farm waste and crop residues should necessarily be given due importance for increasing soil productivity.

Biofertilizers such as *Rhizobium*, *Azotobacter*, *Azospirillum* and blue green algae (BGA) have been in use a long time. Priming is a process of

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seed treatment that refers to combination of seed hydration and inoculation of seed with beneficial organisms to protect seed. It is an ecological approach using either bacteria or fungal antagonists (*Pseudomonas*, *Trichoderma*, *Bacillus* etc.) against the soil and seed borne pathogens. Priming leads to increased vigor, uniform emergence, good stand establishment in many crop plants (Javid *et al.* 2013).

Since rice being cultivated throughout the year, quality seed production plays a vital part to supply seeds in time at reasonable cost. Hence, seed production using organic means is getting momentum now a days. As per the stipulations of International Federation of Organic Agriculture Movements (IFOAM), sowing organic seed has become mandatory for organic agriculture since 2003. With this insight in view, the present study "Effect of organic seed production techniques on yield and quality in rice var. gangavathisona" was conducted.

## MATERIALS AND METHODS

The field experiment was conducted at Seed Technology Research Unit, J-Block, NSP, GKVK, UAS, Bengaluru during *kharij*, 2019. The experimental site was situated at 12° 15' North latitude and 77° 35' East longitudes having an altitude of about 930 m above Mean sea level (MSL). The seeds of Gangavathi Sona were primed with bio priming agents *i.e.*, *Pseudomonas fluorescens* and *Trichoderma harzianum* 1:1.5 dilution for 12 hours. Seeds were then dried in shade and used for sowing. The field experiment was laid out in a Factorial RCBD design with 12 treatments and 3 replications. Fertilizer treatments - N<sub>1</sub>: Control (No Fertilizer and Manure), N<sub>2</sub>: 125 kg Neem + 1250 kg vermicompost per ha +10 kg *Azospirillum* per ha + 10 kg PSB per ha + 10 kg KSB per ha, N<sub>3</sub>: 125 kg Neem + 10 tonnes FYM per ha + 10 kg PSB per ha, N<sub>4</sub>: State recommended dose of fertilizer (100:50:50 kg NPK per ha). Priming treatments - P<sub>1</sub>: Control (No priming), P<sub>2</sub>: Seed priming with *Trichoderma harzianum* (1.5 %) for 12 hours, P<sub>3</sub>: Seed priming with 20 % liquid *Pseudomonas fluorescens* for 12 hours.

## RESULTS AND DISCUSSION

The results of the study revealed that marked difference in growth parameters was observed over different fertilizer treatments. The maximum plant

height (79.52 cm), number of productive tillers (16.20), number of panicles (15.13) and panicle length (19.73 cm) was noticed in inorganic nutrients received treatment N<sub>4</sub> (Urea-217.4 kg, SSP-312.5 kg, MOP-83.33 kg/ha) which is on par with organic treatment N<sub>3</sub> (125 kg neem cake + 10 tonnes FYM + 10 kg PSB per ha)(76.59 cm, 15.50, 14.11 and 18.60 cm respectively) and minimum was recorded under treatment that received no fertilizers (N<sub>1</sub>) (66.39 cm, 12.21, 11.51 and 15.71 cm respectively). This increase in plant height in response to RDF might be primarily due to the improved vegetative growth and supplementary contribution of nitrogen. Similar results were reported by Muhammad *et al.* (2014) in paddy that maximum plant height (131.34 cm) was recorded in application of RDF (150:90:60 NPK kg/ha).

Maximum tiller production is because of availability of more phosphorous. Matsuo *et al.* (1995) reported that it is necessary to apply much P fertilizers to help rice plants to accelerate the phosphate absorption for increased tillering. Kaushik Chakraborty (2011) also reported the similar results in paddy. Maximum panicle length (17.10 cm) was recorded through 100 kg N/ha in paddy cultivar Tulaipanaj. Panicle length is a genetically controlled trait, but sometimes also influenced by availability of nutrients (Matsushima, 1980).

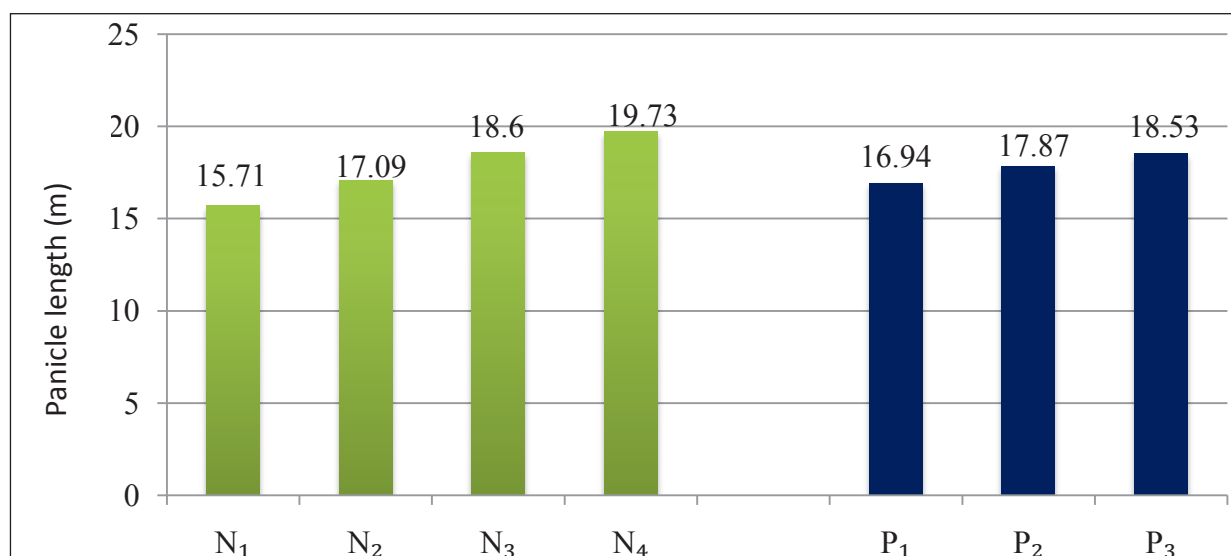
Among the different priming treatments, seed priming with 20% liquid *Pseudomonas fluorescens* for 12 hrs. (P<sub>3</sub>) recorded the highest plant height (75.66 cm), number of productive tillers (15.32), number of panicles per hill (14.52) and panicle length (18.53 cm) while the lowest (71.23 cm, 13.81, 12.63 and 16.94 cm respectively) was recorded in control *i.e.* no priming (P<sub>1</sub>).

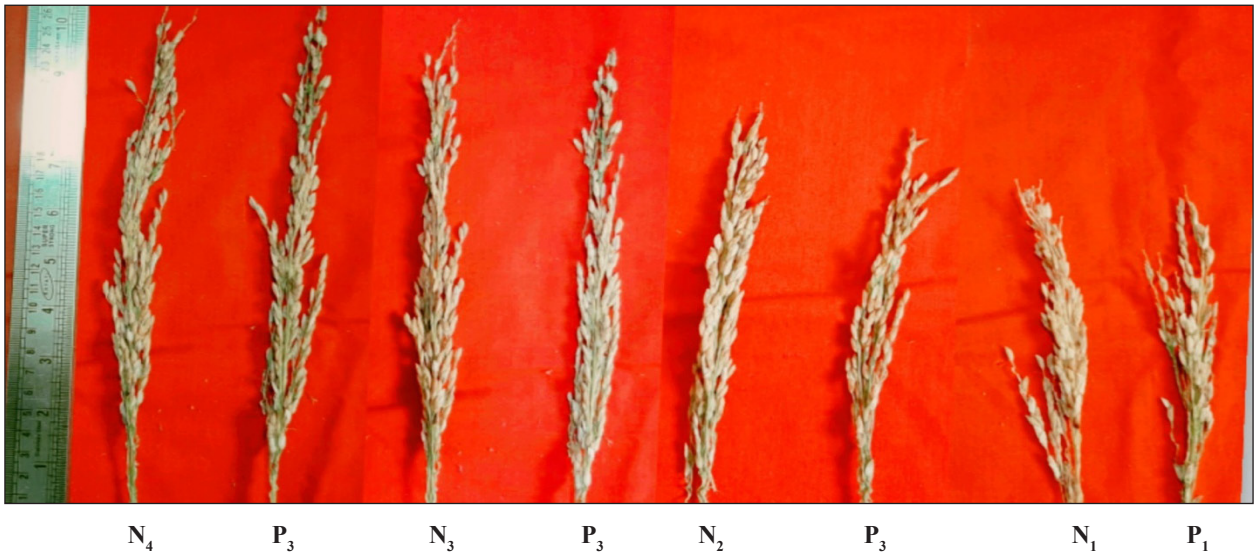
Significant difference was observed for yield parameters over different fertilizer sources. Inorganic nutrients received treatment N<sub>4</sub> (Urea-217.4 kg, SSP-312.5 kg, MOP-83.33 kg/ha) recorded the maximum number filled seeds per panicle (111.81), seed yield per plant (18.81 g) and seed recovery (92.76%) which is on par with organic nutrients received treatment N<sub>3</sub> (125 kg neem cake + 10 tonnes FYM + 10 kg PSB per ha) (110.02, 18.35 g and 91.88 % respectively) and treatment that received no fertilizers (N<sub>1</sub>) recorded the minimum (102.10, 16.85 g and 86.59 % respectively). Chaturvedi (2005) reported that a greater number of tillers, particularly fertile tillers,



**Table 1:** Influence of organic manures and seed priming on growth parameters in paddy var. gangavatisona

Treatments	Plant height at harvest (cm)	Number of productive tillers per hill	Number of panicles per hill	Panicle length (cm)
<b>(A) Fertilizer source</b>				
N <sub>1</sub> : No fertilizers	66.39	12.21	11.51	15.71
N <sub>2</sub> : 125 kg neem cake + 1250 kg vermicompost + 10 kg <i>Azospirillum</i> + 10 kg PSB + 10 kg KSB (per ha)	72.06	14.72	13.77	17.09
N <sub>3</sub> : 125 kg neem cake + 10 tonnes FYM + 10 kg PSB (per ha)	76.59	15.50	14.11	18.60
N <sub>4</sub> : Recommended dose of fertilizers (urea-217.4 kg + SSP- 312.5 kg + MOP-83.33 kg/ha)	79.52	16.20	15.13	19.73
<b>SEm±</b>	1.881	0.476	0.365	0.494
CD (P=0.05)	5.517	1.397	1.070	1.450
<b>(B) Priming treatments</b>				
P <sub>1</sub> : Control (No priming)	71.23	13.81	12.63	16.94
P <sub>2</sub> : Seed priming with <i>Trichoderma harzianum</i> (1.5 %) for 12 hrs	74.03	14.86	13.75	17.87
P <sub>3</sub> : Seed priming with 20% liquid <i>Pseudomonas fluorescence</i> for 12 hrs	75.66	15.32	14.52	18.53
<b>SEm±</b>	1.629	0.412	0.316	0.428
CD (P=0.05)	NS	1.210	0.927	1.256
<b>(C) Interaction (N × P)</b>				
N <sub>1</sub> P <sub>1</sub>	65.00	10.98	10.78	15.23
N <sub>1</sub> P <sub>2</sub>	66.73	12.36	11.11	15.74
N <sub>1</sub> P <sub>3</sub>	67.44	13.30	12.67	16.18
N <sub>2</sub> P <sub>1</sub>	68.86	14.13	13.31	15.87
N <sub>2</sub> P <sub>2</sub>	72.06	14.39	13.59	17.34
N <sub>2</sub> P <sub>3</sub>	75.26	15.15	14.43	18.07
N <sub>3</sub> P <sub>1</sub>	72.26	14.32	12.40	17.48
N <sub>3</sub> P <sub>2</sub>	78.45	16.00	14.83	18.68
N <sub>3</sub> P <sub>3</sub>	79.07	16.20	15.10	19.64
N <sub>4</sub> P <sub>1</sub>	78.82	15.31	14.03	19.20
N <sub>4</sub> P <sub>2</sub>	78.89	16.13	15.47	19.76
N <sub>4</sub> P <sub>3</sub>	80.87	16.66	15.89	20.24
<b>SEm±</b>	3.258	0.825	0.632	0.856
CD (P=0.05)	NS	NS	NS	NS
CV (%)	7.66	9.74	8.03	8.34

**Fig. 1:** Influence of organic manures and seed priming on panicle length in paddy



**Plate 1:** Influence of organic manures and seed priming on panicle length in paddy

**Table 2:** Influence of organic manures and seed priming on yield parameters in paddy var. gangavatisona

Treatments	Number of filled seeds per panicle	Seed yield per plant (g)	Seed recovery (%)
<b>(A) Fertilizer source</b>			
N <sub>1</sub> : No fertilizers	102.10	16.85	86.59
N <sub>2</sub> : 125 kg neem cake + 1250 kg vermicompost + 10 kg <i>Azospirillum</i> + 10 kg PSB + 10 kg KSB (per ha)	107.34	18.23	89.51
N <sub>3</sub> : 125 kg neemcake + 10 tonnes FYM + 10 kg PSB (per ha)	110.02	18.35	91.88
N <sub>4</sub> : Recommended dose of fertilizers (urea-217.4 kg + SSP- 312.5 kg + MOP-83.33 kg/ha)	111.81	18.81	92.76
<b>SEm±</b>	1.94	0.424	1.517
CD (P=0.05)	5.71	1.246	4.541
<b>(B) Priming treatments</b>			
P <sub>1</sub> : Control (No priming)	105.62	17.32	87.42
P <sub>2</sub> : Seed priming with <i>Trichoderma harzianum</i> (1.5 %) for 12 hrs	108.08	18.19	91.06
P <sub>3</sub> : Seed priming with 20% liquid <i>Pseudomonas fluorescense</i> for 12 hrs	109.75	18.68	92.08
<b>SEm±</b>	1.68	0.368	1.314
CD (P=0.05)	NS	1.079	3.854
<b>(C) Interaction (N × P)</b>			
N <sub>1</sub> P <sub>1</sub>	101.47	16.34	84.00
N <sub>1</sub> P <sub>2</sub>	102.15	16.91	87.59
N <sub>1</sub> P <sub>3</sub>	102.70	17.32	88.18
N <sub>2</sub> P <sub>1</sub>	105.25	17.91	87.63
N <sub>2</sub> P <sub>2</sub>	107.47	18.26	89.82
N <sub>2</sub> P <sub>3</sub>	109.30	18.95	91.11
N <sub>3</sub> P <sub>1</sub>	107.02	17.47	88.66
N <sub>3</sub> P <sub>2</sub>	110.13	18.55	92.69
N <sub>3</sub> P <sub>3</sub>	112.93	19.05	94.29
N <sub>4</sub> P <sub>1</sub>	108.75	17.98	89.40
N <sub>4</sub> P <sub>2</sub>	112.59	19.04	94.16
N <sub>4</sub> P <sub>3</sub>	114.09	19.43	94.74
<b>SEm±</b>	3.37	0.736	2.628
CD (P=0.05)	NS	NS	NS
CV (%)	5.42	7.05	5.04



led to higher yields in paddy. Similar results are also obtained by Mamta *et al.* (2014) where application of 100 % RDN through inorganic fertilizers provided maximum seed yield (35.50 kg/ha) compared to control.

Among different priming treatments maximum number of filled seeds per panicle (109.75), seed yield per plant (18.68 g) and seed recovery (92.08%) was recorded in seed priming with 20 % liquid *Pseudomonas fluorescence* for 12 hrs (P<sub>3</sub>) while, minimum number of filled seeds per panicle (105.62, 17.32 g and 87.42 % respectively) was recorded under control i.e. no priming (P<sub>1</sub>).

## CONCLUSION

Organic treatment, 125 kg neem + 10 tonnes FYM + 10 kg PSB (per ha) has recorded the on par results with RDF (urea-217.4 kg + SSP-312.5 Kg + MOP-83.33 kg/ha) treatment with respect to growth parameters viz., plant height, number of tillers per hill, number of panicles per hill and panicle length. Although seed yield parameters were maximum by application of RDF but were on par with organic treatment (125 kg neem cake + 10 tonnes FYM + 10 kg PSB). Hence, use of organics i.e., FYM, compost, green manure, green leaf manure and bio fertilizers should necessarily be given due importance for seed production instead of using chemical fertilizers.

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# *Pseudomonas* Species Potential for Organophosphorus Degradation

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## ABSTRACT

Pesticides and fungicides are also often used to increase food output by protecting crops from rodents and pests that cause damage. These pollutants, as well as residues from medications, antibiotics, and pesticides, infiltrate the human diet, creating a health risk. The microbial degradation of pesticides in soil environments is an important topic in the field of global environmental restoration research and technology. The method and application of pesticide microbial degrading bacteria, notably organophosphorus pesticides, which are difficult to degrade in natural settings, were detailed in this work. The mild and regulated method, environmentally friendly reaction, and high potency of organophosphorus degradation using microorganisms and organophosphorus degrading enzymes appear promising. Various enzymes with the potential to degrade OP include organophosphorus hydrolases (OPH), Methyl parathion Hydrolase (MPH), and CP lyases. The organic phosphate hydrolyser gene opd (organic phosphate degradation) has been isolated from geographically diverse taxa.

## HIGHLIGHTS

- ① *Pseudomonas* species are effective organophosphate degraders with enzymes which degrade organophosphorus pesticides.
- ② Various mechanisms having different enzyme set can degrade organ phosphorus pesticides.

**Keywords:** *Pseudomonas* species, organophosphorus, organophosphorus hydrolases, genetic basis

Gram-negative aerobic bacilli measuring 0.5 to 0.8  $\mu$ m by 1.5 to 3.0  $\mu$ m, *Pseudomonas* species are gram-negative. A single polar flagellum is responsible for motility. Biochemical and DNA hybridization assays are used to distinguish species. *P. aeruginosa*, *P. fluorescens*, *P. putida*, *P. cepacia*, *P. stutzeri*, *P. maltophilia*, and *P. putrefaciens* are among these bacteria. *P. mallei* and *P. pseudomallei* are the only two species that cause glanders and melioidosis in humans. The type III secretion system (TTSS) is a unique cellular intoxication mechanism that *Pseudomonas* species use to transport toxins into eukaryotic cells and to remove or change host innate defense (Roy-Burman A *et al.* 2001).

Pesticides are chemicals that are used to keep pests at bay. Herbicides, insecticides, nematicides,

molluscicides, piscicides, avicides, rodenticides, bactericides, insect repellents, animal repellents, microbicides, and fungicides are some examples. Pesticides are substances that help increase crop output by killing pests, insects, mollusks, and nematodes.

Pesticides used in excess quantity to kill weeds and pests have become unbearable in agriculture, enhancing production but also producing a slew of environmental issues, including non-target organism consequences (Thiour-Mauprivez C *et al.* 2019).

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Pesticides environmental destiny is influenced by both biological and abiotic processes (Fig. 1). Pesticides degrade in the environment primarily through the action of soil microorganisms such as bacteria and fungi. The breakdown of chemicals by microbes or their enzymes into smaller, less hazardous compounds is known as biodegradation.

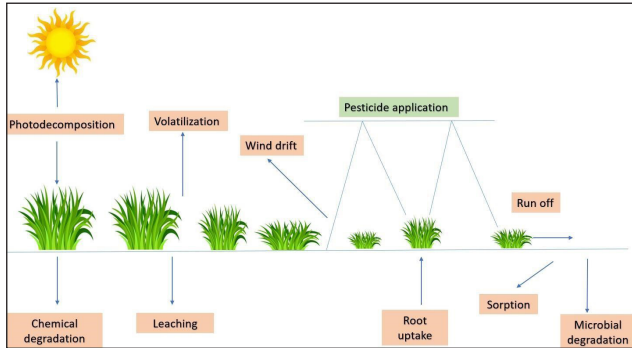


Fig. 1: The fate of pesticides in the environment

Bacterial strains can naturally produce substantial amounts of reactive oxygen species (ROS) when they are exposed to harsh environmental conditions. Pesticides, for example, create oxidative stress by producing reactive oxygen species (ROS), which interact with the cell membrane and promote lipid peroxidation. Bacteria have created response systems to safeguard membrane integrity, such as adjusting the activity of antioxidant enzymes, to prevent or reduce these types of imbalances (Martin *et al.* 2011; Lemire *et al.* 2017). By secreting extracellular polymeric substances (EPS) and building biofilms, bacteria can become resilient to oxidative stress (Tolker- Nielsen *et al.* 2015). Different levels of susceptibility and sensitivity of bacterial communities to stressors can result in changes in their diversity and sensitivity.

Because of its metabolic and physiological diversity, the genus *Pseudomonas* stands out for its tolerance to chemical stressors, and it may be isolated from soil, freshwater, biofilms, and other sites, with potential for use in bioremediation (Venturi *et al.* 2006; Karami *et al.* 2016; Melo *et al.* 2016). (Lima *et al.* 2020) gathered bacteria that could tolerate several herbicides, including *Pseudomonas* sp. CMA 6.9, which was tested in this study. This strain was obtained from tank water used to wash pesticide packaging that contained the Heat® herbicides. Non-target-site resistance (NTSR) includes metabolic processes that have been described in plants, such

as reactions involving esterase enzymes, GSTs, uridine 5'diphospho-glucosyl transferases, and cytochrome P450s (Jugulam *et al.* 2019; Gaines *et al.* 2020).

### Organophosphorus compounds:

Phosphoric acids and their derivatives produce organophosphorus compounds (OPCs), which are natural molecules with at least one carbon-phosphorus bond (Fig. 2). Pentavalent phosphorus-containing compounds are commonly used in commercial and environmental applications. Phosphorus substituents in phosphoric acid esters play an important role in toxicity (Balali-mood *et al.* 2014).

Phenoxy, cyanides, or thiols, amides, or esters of phosphonic acids, phosphinic acids, phosphoric acids, or thiophosphates with a more natural side chain of thiocyanic acid groups (Kumar *et al.* 2016). Phosphonothioates are some OPCs, and the phosphonofluoridates classes include nerve poison, sometimes known as chemical war chemicals (Gupta *et al.* 2006).

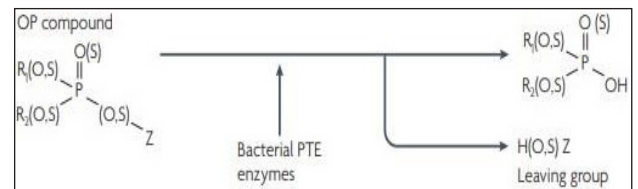


Fig. 2: Structure of organophosphorus compound

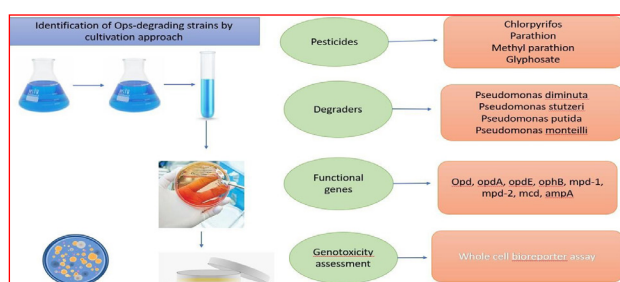
Organophosphorus compounds, glyphosate, chlorpyrifos, parathion, methyl parathion, diazinon, coumaphos, monocrotophos, fenamiphos, and phorate are widely used.

### Degradation of Organophosphorus by specific *Pseudomonas* species

Pesticide breakdown and detoxification are mostly accomplished through microbial biodegradation. Microorganisms can have a big impact on the long-term viability of most insecticides in the soil. When organophosphate is released into the environment, it is determined by a variety of environmental factors as well as microorganism breakdown. Furthermore, several organophosphorus insecticides and their metabolites are hazardous and require decomposition in contaminated soil. The fundamental reason for the elimination of these

pesticides is that they are degraded by microbes. However, knowing all of the physiological, microbiological, ecological, biochemical, and molecular elements involved in the conversion of pollutants is required when using microorganisms in bioremediation (Iranzo *et al.* 2001).

Some microorganisms, fungi, and cyanobacteria have been isolated that can utilize OP chemicals such as carbon, nitrogen, or phosphorus assets. The identification of microbes that can metabolize OP chemicals has attracted a lot of attention. Because in situ detoxification procedures using such microbes are environmentally friendly. Local microbial populations in a few infected areas have evolved over time to conform to those contaminants.



**Fig. 3:** Microbial degradation of organophosphorus

Plants, soils, water, animals, flooded soils, anaerobic cultures, and soil microorganisms have all been studied for a microbial breakdown of organic phosphates. Bacteria from flooded soil were shown to hydrolyze a variety of organophosphorus insecticides. For biotechnological purposes, it's critical that bacterial degraders be able to break down all or nearly all OP substances. A few relevant species have been isolated; for instance, *Pseudomonas diminuta*, which has been isolated from an enrichment lifestyle in the United States, can cleave all recognized OP bonds.

(Kavi *et al.* 2012) isolated pesticide-degrading bacteria, and the isolated bacterial isolate was identified as *Pseudomonas fluorescens*. The growth of these pesticide-degraded isolates was tested using two distinct pesticides, malathion and parathion with minimum saline, and was found to be the same in all three bacterial isolates.

The most important step in detoxifying OP compounds is hydrolysis to make them more biodegradable (Kumar *et al.* 1996). The mechanism of hydrolysis and its kinetic properties are well known (Brown *et al.* 1980; V Lewis *et al.* 1988;

Dumas *et al.* 1989; DP Dumas *et al.* 1990; ML Ortiz *et al.* 2003). PTE can also be used to clean up polluted environments. However, in previous laboratory experiments using cultures in aqueous media, this enzyme was observed to have no effect on some OPs, and the phosphate used to form the organic phosphate ester. It shows the peculiarities of its dependent activities. The bacteria would convert organic macromolecules into small non-toxic molecules, thus avoiding secondary pollution. Studies have shown that mineralization and co-metabolism were the main mechanisms for the further degradation of pesticides and their intermediate product (Table 1). A list of *Pseudomonas* species capable of degrading these compounds is presented in the following tables.

**Table 1:** *Pseudomonas* species degrading organophosphate compounds

Organophosphates	<i>Pseudomonas</i> spp.	Mode of degradation	Reference
Chlorpyrifos	<i>Pseudomonas diminuta</i>	Co-metabolic	(Serdar <i>et al.</i> 1982)
Chlorpyrifos	<i>Pseudomonas resinovarans</i> strain AST2.2	Co-metabolic	(Sharma <i>et al.</i> 2016)
Parathion	<i>Pseudomonas stutzeri</i>	Co-metabolic	(Daughton <i>et al.</i> 1977)
Methyl parathion	<i>Pseudomonas putida</i>	Catabolic (C)	(Rani <i>et al.</i> 1994)
Glyphosate	<i>Pseudomonas sp.</i>	Catabolic (P)	(Kertesz <i>et al.</i> 1994)
Coumaphos	<i>Pseudomonas monteilli</i>	Co-metabolic	(Horne <i>et al.</i> 2002)
Fenamiphos	<i>Pseudomonas putida</i>	Catabolic (P)	(Eisenmaan <i>et al.</i> 2002)
Diazinon	<i>Pseudomonas spp.</i>	Co-metabolic	(Rosenberg <i>et al.</i> 1979)

### Few pesticides and their modes of degradation

#### Chlorpyrifos

Chlorpyrifos is one of the maximum generally used pesticides, it's powerful in opposition to an extensive variety of pests on economically vital crops. In 2007, America used approximately eleven million kilos of chlorpyrifos, extra than another organophosphorus pesticide (Grube *et al.* 2011) to combat in opposition to mosquitoes (larvae and adults), flies, and numerous soils, leaves, and family



pests. It is likewise used to govern ectoparasites in farm animals and sheep. It has low solubility in water ( $1.18 \text{ mg L}^{-1}$ ) however is without difficulty soluble in maximum natural solvents. According to WHO (2009), that is an addictive compound (magnificence II) with a median deadly dose (LD50) in mammals of approximately 32-a thousand  $\text{mg kg}^{-1}$ . The environmental destiny of chlorpyrifos has been substantially studied (Kim *et al.* 2009). Soil decomposition entails each microbial hobby and chemical hydrolysis. The half-existence of chlorpyrifos withinside the soil is ready at 38 days, whilst the half-existence in water (hydrolyzed half-existence) is two 118 days (Kegley *et al.*, 2014).

*Pseudomonas putida* MAS-1 is reported to be more efficient in chlorpyrifos degradation by a rate of 90% in 24 h among the *Pseudomonas* genus.

Chlorpyrifos is co-metabolized and metabolically destroyed by microbes isolated from a variety of matrices, including agricultural soil, industrial sludge, activated sludge, and wastewater (Li, X *et al.* 2008; Chishti *et al.* 2013). *Pseudomonas putida*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Pseudomonas nitroreducens*, and *Pseudomonas fluorescens*, for example, can be isolated from agricultural soils and infected sewage in various places and remain relatively green for chlorpyrifos biodegradation. It has been demonstrated (Bhagobaty *et al.* 2010; Maya *et al.* 2011; Latifi *et al.* 2012; Sasikala *et al.* 2012).

## Parathion

The pesticide parathion (O, O-diethyl-O-p-nitrophenyl phosphorothioate) is one of the most dangerous pesticides that the US Environmental Protection Agency has ever reported (EPA). Many humans and non-target species have died as a result of extreme toxicity at low levels in some underdeveloped countries (McConnell *et al.* 1999). Because of its widespread use and easy detection of its hydrolysis product, microbial degradation of parathion has gotten a lot of interest among organophosphorus compounds (nitrophenol). In biologically active soils, parathions disintegrate quickly. With increasing concentrations of added parathion, a proportional rise in the number of bacteria in the soil was found. In the rhizosphere of rice seedlings, flooding soil conditions enhanced parathion hydrolysis and the release of  $^{14}\text{CO}_2$  from

ring-labelled parathion. Parathion is retained in body fat and released slowly into the bloodstream, extending the harmful effect.

It breaks down parathion into nitrophenol, which will be used as a carbon or nitrogen source. *P. stutzeri*, which can hydrolyze parathion, was later discovered. Nitrophenol, on the other hand, was metabolized by every other bacterium. *Pseudomonas* spp. can hydrolyse a variety of organophosphorus compounds, including parathion, and ionic fission products can be employed as the most convenient source of phosphorus.

## Methyl parathion

Parathion Methyl, often known as methyl parathion, is an organophosphate insecticide that contains an organothiophosphate group. It resembles Malathion in terms of structure. The chemical structures of methyl parathion and ethyl parathion are identical, with the exception that the ethyl group in the parathion's P chain is substituted by a methyl group. *Pseudomonas* has been found in soil and sodium alginate beads degrading methyl parathion. Nitrophenol can be used as the only carbon and nitrogen source by *Pseudomonas* A3. As a sole source of carbon, this isolate can also use a wide range of aromatic compounds (Zhongli *et al.* 2002). Methyl parathion also produces nitrophenol as a hydrolysis product.

## Glyphosate

Herbicide glyphosate (N (phosphonomethyl) glycine) is used all over the world. It belongs to the phosphonate binding group, which is distinguished by the presence of a straight carbon-phosphorus (CP) bond. CP bonds are substantially more resistant to non-biodegradation in the environment than OP bond analogs because they are chemically and thermally stable (Hayes *et al.* 2000). Glyphosate works by inhibiting the enzyme 5-enolpyruvate-3-phosphate synthase, which catalyses the synthesis of aromatic amino acids in plants. Glyphosate has a half-life of 30170 days, making it a moderately long-lasting pesticide (Tomlin *et al.* 2000).

The most important conversion process that governs its permanence in the soil is microbial breakdown. Glyphosate mineralization has been linked to both





microbial activity and biomass in the soil. The primary metabolite amino methyl phosphonic acid is generated during the microbiological breakdown of glyphosate, eventually leading to the creation of CO<sub>2</sub>, phosphates, and water (Araujo *et al.* 2000). Several bacterium species that may digest glyphosate co-metabolically or as a source of phosphorus have been discovered from previously treated and untreated environments. *Pseudomonas* species capable of decomposing glyphosate have been identified.

### Organophosphorus degrading by microbial enzymes

Several studies also claimed that the microbial enzymes are also effectively degrading the effectiveness of organophosphoric pesticides. Few studies are discussed and summarized in below paragraphs.

A number of microbial species have identified and characterised enzymes capable of hydrolyzing organophosphorus substances. Because the hydrolysis of organophosphorus compounds reduces the toxicity of mammals by orders of magnitude, this phase is also known as detoxification. Phosphotriesterase (PTE), which was first found in *Pseudomonas diminuta* MG and can hydrolyze a large range of synthetic Organophosphates, is one of the most important enzymes (Serdar *et al.* 1989; Mulbry *et al.* 2000).

Phosphotriesterase (PTE) is a set of enzymes found in microbes, animals, and plants that can degrade OP compounds. The bacterium PTE comes in three varieties. This enzyme is known as an organophosphorus hydrolyase (OPH) (Mansee *et al.* 2005), and it catalyses the hydrolysis of various organophosphates by cleaving the PO and PS bonds of these OPs. (Ang *et al.* 2005). Organophosphorus hydrolases (OPH) and methyl parathion hydrolases are two of the most investigated enzymes in biological sciences (MPH).

The enzymatic reaction known as microbial degradation was used more frequently because pesticides were primarily used as a microbial nutrient, and eventually decomposed into small molecules such as CO<sub>2</sub> and H<sub>2</sub>O, which included that the compound entered the microorganism's body through a specific route, and then passed

through a series of physiological and biochemical reactions involving various enzymes, the pesticide was completely degraded or broken down.

*Pseudomonas* spp. strain ADP, for example, utilized atrazine as its only carbon source, and three enzymes were involved in the first few steps of atrazine breakdown. AtzA was the first enzyme discovered, and it catalysed the hydrolysis dichlorination of atrazine to non-toxic hydroxyl atrazine. It was an important enzyme in the biological degradation of atrazine. The dehydrochlorination of hydroxy atrazine to create N-isopropyl cyanuric amide was catalysed by the second enzyme, AtzB. AtzC was the third enzyme, and it catalysed the formation of cyanuric acid and isopropyl amine from N-isopropyl cyanuric amide. Atrazine was eventually broken down into CO<sub>2</sub> and NH<sub>3</sub> (De Souza *et al.* 1996; Wackett *et al.* 2002).

Degrading enzymes were typically more resistant to adverse environmental conditions than microbial cells capable of producing such enzymes, and their degradable efficiency was substantially higher than that of microorganisms, especially at low pesticide concentrations. Using degrading enzymes to detoxify the environment that has been contaminated by pesticides would be a more effective method. However, due to non-degeneration and soil adsorption in the soil, the degrading enzyme was quickly inactivated, making it difficult to maintain the degradable activity for an extended period of time. Degrading enzymes have also been limited in practise due to low mobility of the enzyme in the soil and other variables (Arbeli *et al.* 2007; Huong *et al.* 2008).

The degradable methods included oxidation (hydroxylation reactions such as aromatic hydroxylation, aliphatic hydroxylation, N-hydroxylation, epoxidation, N-oxidation, P-oxidation, S-oxidation, oxidative dealkylation, oxidative dehalogenation, and oxidative deamination), reduction (nitro group reduction, quinone reduction, and reductive dehalogenation), and hydrolysis (some esters such as thiophosphate. The effect of individual enzymes and the mechanism for organophosphorus insecticides are briefly summarised here.

## Organophosphorus hydrolase (OPH)

Organophosphorus hydrolase (OPH) is a bacterial enzyme that can detoxify a wide range of organophosphorus (OP) agents by hydrolyzing various phosphorus-ester bonds (P-O, P-F, P-CN, and P-S). OP compounds are toxic molecules used primarily as pesticides and nerve agents.

Organophosphorus hydrolases have been isolated from several bacteria. Among the bacterial enzymes, *P. diminuta* has the widest range of substrate specificity. Organophosphorus hydrolases have a wide range of substrate specificities. It hydrolyzes P-O, P-F, and P-S bonds to varying degrees. The lowest specificity applies to PS bindings. However, this enzyme does not catalyze the cleavage of carbonyl groups as seen in nitrophenyl acetate. The effect of metal substitution on the catalytic activity of OPH is that the purified OPH is depleted of natural metals (Zn) and reconstituted with various divalent cations such as Co, Cd, Cu, Fe, Mn, and Ni.

The bacterial enzyme organophosphorus hydrolase (OPH) has been found to break down a wide range of neurotoxic organophosphate nerve poisons. The efficiency of degradation, on the other hand, varies greatly, from highly efficient with paraoxon to relatively slow with methyl parathion. The activity of OPH towards poorly degraded substrates was fine-tuned and enhanced via sequential cycles of DNA shuffling and screening.

## Methyl parathion hydrolase (MPH)

MPH is found in many phylogenetically unrelated bacteria and is active against many OP compounds, but has less substrate diversity than OPH. The crystal form of MPH (a member of the  $\beta$ -lactamase superfamily) of the genus *Pseudomonas*. WBC 3 has been resolved. MPH is a dimer in which each subunit contains a hybrid dikaryon mixed zinc centre. However, MPH is not always homologous to other PTEs, but many PTEs can degrade methyl parathion (Dong *et al.* 2005). For the quick pre-screening of possible variations with improved methyl parathion hydrolysis, a solid-phase top agar approach based on the detection of the yellow product p-nitrophenol was devised. Several better variations were discovered after two rounds of DNA shuffling and screening. 22A11 is a mutant that hydrolyzes methyl parathion 25 times faster than

the wild type. We believe we can easily extend this method to create other OPH variants with improved activity against poorly degraded pesticides such as diazinon and chlorpyrifos, as well as nerve agents such as sarin and soman, based on our success with directed evolution of OPH for improved hydrolysis of methyl parathion.

## Genetic basis of organophosphorus degradation

The first organophosphorus depletion (opd) gene is found in *P. diminuta* and has shown to be present on a plasmid (Table 2). The plasmid size was 66 kb and was named pCMS1. The 1.5 kb BamHI fragment, which has restriction sites specific to Sall, PstI, and XhoI, has been shown to encode this enzyme by cloning into various plasmids and broad-spectrum cloning vectors. Sequencing of the opd gene showed that the genes of both bacteria have the same sequence (Harper *et al.* 1988).

**Table 2:** List of genes, their origin, vector, and gene products involved in the degradation of organophosphorus compounds

Gene	Organism	Location	Encoded enzyme	References
Opd		Plasmid	OPH	(Serdar <i>et al.</i> 1989; Chaudry <i>et al.</i> 1988)
	<i>Pseudomonas diminuta</i> ,	Plasmid	OPH	
	<i>Pseudomonas</i> sp.			
hocA	<i>Pseudomonas Monteilli</i>	Chromosome	ND	(Horne <i>et al.</i> 2002)
glp A&B	<i>Pseudomonas pseudoaeruginosa</i>	Chromosome	C-P lyase	(Penalzoza-Vazquez <i>et al.</i> 1995)

## Major modes and pathways for microbial degradation of organophosphate pesticides (mode of action):

1. Adsorption
2. Photodegradation
3. Hydrolysis
4. Enzymatic degradation (enzymatic hydrolysis)

### 1. Adsorption

Adsorption reduces the loss of organophosphate insecticides by volatilization or leaching. Van



der Waals forces, dipole-dipole interactions, hydrogen bonding, and ion exchange are some of the physicochemical forces involved in the adsorption process by soil particles (Smolen *et al.* 1998). However, there is less data on ionizable pesticide adsorption, and more research is needed to understand background mechanisms and predict the nature of pesticide-soil interactions, because these phenomena affect other processes that determine the compound's final fate, such as chemical, photochemical, and microbiological decomposition, volatilization, plant uptake, and diffusion (Beltran J *et al.* 1995). Organophosphate pesticides behaviour in nature is significantly influenced by adsorption and the associated reduced mobility in soils. Many factors determine the degree of adsorption as well as the rate and extent of final degradation, including pesticide solubility, volatility, charge, polarity, molecular structure, and size. The process of adsorption by soil particles can either slow or speed up the decomposition of organophosphate pesticides by isolating the pesticide from the enzymes that break it down. The adsorption mechanism is aided by abiotic hydrolytic degradation (Smolen *et al.* 1998).

## 2. Photodegradation

Various organophosphate insecticides have been examined for photodegradation (Lacorte S *et al.* 1994; Derbalah *et al.* 2004). The rate of breakdown is fast, and the degradation products range from the oxidized P=S bond to those produced by isomerization of the initial organophosphate insecticide. In both the aqueous and gas phases, photolysis could be a significant degradation mechanism.

## 3. Hydrolysis

The most well-studied breakdown route for organophosphate insecticides is hydrolysis. As a result, this section concentrates on some of the most exciting results about organophosphate pesticide hydrolysis mechanisms. The cleavage of the P-S bond (in the case of phosphorodithioates and phosphorothioates) or the P-O bond (in the case of phosphorothioates) results in the best product (Lai *et al.* 1995). (Wang and Hoffman *et al.* 1991) discovered that alkaline hydrolysis is the most common mechanism for malathion,

which is consistent with a previous laboratory work by (Wolfe *et al.* 1977). Malathion has only been discovered to have sluggish biological and photochemical degradation, but parathion has been proven to have significant biological degradation. Only photolysis and alkaline hydrolysis are secondary routes for parathion breakdown. Copper-catalyzed chlorpyrifos hydrolysis is one of the breakdown pathways.

## 4. Enzymatic degradations (enzymatic hydrolysis)

The use of enzymes to break down pesticides has sparked a lot of curiosity. Many aquatic organisms are known to produce enzymes capable of hydrolyzing a wide range of organophosphate insecticides. Organophosphorus acid anhydroses are another name for these enzymes, which have also been called paraoxonase, esterase, phosphotriesterase, diisopropyl fluorophosphatase, somanase, and parathion hydrolase. The organophosphorus acid anhydroses' natural substrates are unknown. These enzymes are capable of hydrolyzing a wide range of organophosphorus acetylcholinesterase inhibitors (Liu *et al.* 2001). Parathion hydrolase, an enzyme produced from an overproducing strain of *Pseudomonas diminuta*, hydrolyzes the phosphate ester link in the organophosphate pesticide molecule, resulting in a 100-fold decrease in toxicity (Havens and Rase *et al.* 1991). The use of hydrolase and related genes in studying the complicated interaction of bacteria with pesticides can help bioremediation efforts by improving our understanding of the biodegradation process.

## Future prospect

Because of its extensive usage as a pesticide and significant toxicity to mammals, the breakdown of organophosphorus compounds has gotten a lot of attention. Numerous bacteria capable of mineralizing or co-metabolizing organophosphorus substances have been identified and described. Some microbes can degrade a wide range of chemicals, whereas, others can only break down one or a few structurally related organophosphorus compounds. This is due to the fact that hydrolysis reduces the toxicity of organophosphorus compounds in mammals by several orders of magnitude. The three-dimensional structure of the organophosphorus



enzyme OPH has been established, as well as its catalytic activity. Site-directed mutagenesis has been used to improve the catalytic activity of weak substrates while lowering enzyme stereoselectivity.

The development of high-efficiency pesticide degradation bacteria, the cultivation of mixed bacteria, the immobilization of degrading bacteria, the study of pesticide-degrading fungi, and the quantitative study of pesticide biodegradation models were the main research directions in microbial pesticide degradation. Researchers began to transition to the building of efficient engineering bacteria in recent years, with the development of genetic engineering and molecular biology on the one hand, and applied the gene recombination technique on the other. The goal was to increase the expression level of specific proteins or enzymes in order to improve degradation efficiency, which would solve the problem of some enzymes in the environment not being able to be stabilized and retain a high level of activity. In short, there was an effective method to eliminate pesticide pollution, which was using microbial agents or fertilizer preparation applied in polluted environment.

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