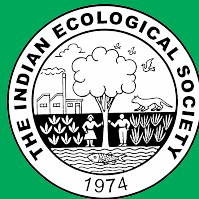


INDIAN  
JOURNAL OF  
*ECOLOGY*

Volume 52

Issue-1

February 2025



THE INDIAN ECOLOGICAL SOCIETY

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# Oldeman's Agroclimatic Zone Shifting Analysis and Suitability of Agricultural Land in Bali

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**Abstract:** Due to climate change, the land suitability for several commodities no longer be the same. Agricultural conditions in Bali province will be expected to face uncertain climate patterns in the future. Therefore, the climate information required by farmers. By knowing the information, they expected able to suit their cultivation planning to the latest climate types information. One of climate information is climate types classification presented as Oldeman's Agroclimatic Zone Map. It resulted by identification of climate types shifting areas. Areas where the wet or dry months become shorter or longer will cause the cultivation period for a commodity to be disrupted, which can reduce crop yields. The research regarding climate types shifting is important to find out which areas experienced in shift and what type of it. The aim of the research is to analyze Oldeman's climate types shifting every decade in the 1991-2020 period to determine the suitability of land to climate for paddy (*Oryza sativa*), corn (*Zea mays*), soybean (*Glycine max.*), chili (*Capsicum annum*) and shallot (*Allium oscolonicum*) in 58 locations to represent all sub-districts in Bali. The results showed that 49 (84.48%) locations experienced shifting.

**Keywords:** Bali, Climate types, Oldeman, Precipitation, Shifting

As one of climatic factor, precipitation influences the success in harvest. Heavier in its intensity has the potential to destruct agricultural land, while very low precipitation results the plantation unable to produce optimally due to limited water availability. Such precipitation condition for recent years have resulted in changes in the number of wet and dry months so that the climate types in several region changed. Grigorieva et al. (2023) stated because agricultural productivity depends on weather and climate and is highly dependent on climatic stability, climate change poses various challenges to agricultural activities. This change means that the level of land suitability for several agricultural commodities is no longer the same, many efforts required to identify areas experienced climate types shifting so that when planning agricultural activities, the commodities to cultivate expected compatible with the latest climate information in the area. The problem of shifting had an impact on decreasing of harvest. The shifting, either in temporary (tended to be wetter or drier, variable) or permanently (wetter, drier) indicated change in the number of wet and dry months, resulted in new climatic patterns that had an impact on productive plants. Pradana and Sesanti (2018) showed that knowledge about climate in the form of its characteristics and predictions was very important so that farmers could determine suitability for agricultural land.

The World Meteorological Organization /WMO (2018)

stated that normal climatological standards calculated every 10 years for 30 years in the initial period of each decade from years ended with the number one (1981-2010, 1991-2020, etc.), and normal recalculations every 10 years required a large data set that used normal precipitation as a reference. The number of rain stations were far apart due to topographic conditions in some areas of Bali, the precipitation data unable to represent condition in some areas so that the precipitation information produced can be inaccurate. Current remote sensing technology makes it possible to monitor precipitation using satellite imagery in the form of estimation data. One of satellite data with a very high resolution is CHIRPS (Climate Hazards Group InfraRed Precipitation with stations) which developed by the United States Geological Survey and the University of California, Santa Barbara. This research uses CHIRPS monthly precipitation data for the period 1991-2020 in Bali. Research using satellite rainfall estimation data was conducted by Noor et al. (2015) using TRMM to create a map of the Oldeman's Agroclimatic Zone and to analyze agricultural resource management. The results of study showed that based on TRMM satellite data, the Agroclimatic Zone in South Kalimantan divided into five: B1, B2, C1, C2, and D1. Irmawan et al (2024) validated the accuracy rate of monthly precipitation CHIRPS data using the correlation coefficient and RMSE to update Oldeman's agroclimatic zone map.

Determination of land suitability in the research based on monthly precipitation using Oldeman's method. According to Wahyunto et al. (2016), the result of Oldeman's method can be used to determine the class of land suitability. Precipitation factor as one of the limiting factors in plant processing and production as well as plays an important role in determining land suitability such as the number of wet and dry months. An uncertainty in seasonal patterns make it difficult for farmers to plan and harvest their commodities. This shifting has increased the scale of vulnerability in agricultural sector, included in Bali. Government needs to give attention for these shifting. The endorsement for the success of agricultural planning can be implemented in several ways, one of them is by providing Oldeman's Agroclimatic Zone Map. This mapping aimed to support the government and farmers in agricultural planning which areas have a climate that is suitable to cultivate certain crops, the classification of which based on suitability of rainfall characteristics to soil.

#### MATERIAL AND METHODS

This classification carried out by calculating the average of monthly precipitation data for January during 1991-2000 period. The data from February to December also calculated to get the average of wet, humid and dry month. A month considered as wet (WM) if the precipitation  $\geq 200$  mm, moist month (MM) if precipitation between 100-200 mm, and dry month (DM) if precipitation  $\leq 100$  mm. The similar procedure carried out to create climate types during the 2001-2010 and 2011-2020 periods, so that types will be obtained for three periods. The making of Oldeman's Agroclimatic Zone criteria based on division of letters from A to E, and numbers, from 1 to 5. Letter A represented of more than nine wet months consecutively, B for consecutive wet months between 7-9 months, C for consecutive wet months between 5-6 months, D for consecutive wet months between 3-6 months, E for consecutive wet months of  $<3$  months. The Sub Division represented consecutive dry months number in a year (Table 1, 2).

The results of climate types classification every decade during 1991-2020 period would produce climate types shifting in each location. The shifting classified into six types:

- a. Wetter; when during three periods the types tended to increase;
- b. Tended to be wet; when the types of first and second periods had the same but the types in the last period was higher.
- c. Constant; when during three consecutive periods the types did not change.
- d. Tended to be dry; when types in first and second periods

were the same but the types in third period was lower.

- e. Drier; when during three periods the types tended to decrease.

- f. Varied; when the types in second period was higher or lower than the first and third;

In this research, the limiting factor was monthly precipitation. Land suitability classified into four items (Wahyunto et al, 2016):

- a. S1 (very suitable) when the land did not have any significant or real limiting factors for sustainably use, or the limiting factors did not dominant and did not significantly reduce the land productivity.
- b. S2 (quite suitable) when the land has limiting factors, and these factors would affect to its productivity, it required additional input. The barriers usually could be overcoming by farmers themselves.
- c. S3 (marginal) when the land had a dominant limiting factor, and this factor would affect to its productivity, it required more additional input than land classified as S2. The way to overcome the factor needed high capital, farmers needed assistance to cope with.
- d. N (not suitable) when the limiting factors very dominant or difficult to overcome.

Precipitation was one of climatic factors to confine the process of plant to grow and production as well as played important role in determining land suitability such as wet and dry months. Classification of land suitability for agricultural commodities based on consecutive wet and dry months as presented below.

The S1 classification or very suitable for paddy stated if range of consecutive wet months between 6-8 months, corn (3-5 months), soybean (2-4 months), and chili (5-6 months). Another example is suitability for shallot based on consecutive dry months in a year. Suitability for shallot determined as S1 if the number of dry months is between 4-6 months; S2 ( $>6$  months); S3 (between 2 and  $<4$  months); and N if the number of dry months  $<2$  months. Shallot will be more suitable to cultivate during dry season.

#### RESULTS AND DISCUSSION

Process of grouping monthly precipitation data by Oldeman's method during the 1991-2000, 2001-2010, 2011-2020 periods produced the Oldeman's agroclimatic zone (Table 5).

The shift either temporarily (tended to be wet, tended to be dry, varied) or permanently (became wetter or drier) indicated a change in number of wet and dry months, resulted a new climate patterns that had an impact on productivity. These conditions affected to land suitability and cultivation patterns for several commodities. Regions shifted in varied

types generally referred as climatic variability. Regions where types were constant, basically had the same suitability class as in previous period, but even though during these three periods the types remained the same. Based on above table, Bali province periodically experienced climate types shifting, on which in 1991-2000 shown by following map.

During 1991-2000 period there were seven types, B2 (two locations), B3 (11), C3 (20), D3 (13), D4 (1), E3 (6) and E4 (5). The type that might only be able to cultivate paddy crop once

**Table 1.** Oldeman's classification of agroclimatic zones

Zone	Climate types	Consecutive wet months	Consecutive dry months
A	A1	10-12	0-1
	A2	10-12	2
B	B1	7-9	0-1
	B2	7-9	2-3
	B3	7-9	4-5
C	C1	5-6	0-1
	C2	5-6	2-3
	C3	5-6	4-6
	C4	5-6	7
D	D1	3-4	0-1
	D2	3-4	2-3
	D3	3-4	4-6
	D4	3-4	7-9
E	E1	0-2	0-1
	E2	0-2	2-3
	E3	0-2	4-6
	E4	0-2	7-9
	E5	0-2	10-12

Source: Alfiandy et al (2021)

a year, depended on availability of irrigation, distributed in outermost, especially in the western and southeastern parts.

In 2001-2010 period there were 11 types, B1 (1), B2 (3), B3 (3), C2 (12), C3 (5), D1 (4), D2 (4), D3 (16), D4 (5), E3 (2) and E4 (3). The period characterized by the addition of four types. Above map shows a shift either in number of wet and dry months or the percentage of types. Dry areas distributed in outside parts and became larger than before.

In 2011-2020 period there were 10 types, B2 (1), C2 (12), C3 (8), D1 (1), D2 (9), D3 (12), D4 (9), E2 (2), E3 (1) and E4 (3). Areas of wet types still distributed in central and southwest parts. The types in the period indicated types shifting happened. In this period, climate types B1 and B3 had unavailable, but there was E2 with 0-2 in consecutive wet months and 2-3 in consecutive dry months.

The differences in number and distribution of Oldeman's types during 3 consecutive periods resulted a trend shifting, there were 2 locations (3.45%) have become wetter, 9 (15.52%) tended to be wet, 9 (15.52%) constant, 18 (31.03%) to be dry, and 5 (8.62%) drier. The classification then updated using monthly precipitation data during the 1991-2020 period resulted in the Oldeman's agroclimatic zone classification presented on Figure 4. Land suitability classification for paddy, corn, soybean, shallot and chili in this study obtained

**Table 3.** Land suitability classification by climate for paddy, corn, soybean and chili based on consecutive wet months

Class	S1	S2	S3	N
Paddy	6 - 8	4 - <6	2 - <4 or >8 - 10	<2 or >10
Corn	3 - 5	<3 or >5 - 7	>7 - 8	>8
Soybean	2 - 4	>4 - 6	>6 or <2	-
Chili	5 - 6	3 - <5 or >6 - 8	<3 or >8	-

Source: Wahyunto et al 2016

**Table 2.** Oldeman's classification of agroclimatic zones with explanations

Climate types	Explanations
A1, A2	Suitable to cultivate paddy continuously but production become less because usually the radiation intensity becomes low throughout the year.
B1	Suitable to cultivate paddy continuously through good planning to start cultivating on planting season.
B2, B3	High production during the dry season.
C1	Only able to cultivate paddy twice a year with short-term paddy variety, and when the dry season quite short, it enough to cultivate other secondary crops.
C2, C3, C4	Only able to cultivate paddy once and other secondary crops twice a year
D1	Only able to cultivate paddy once a year, and other secondary crop cultivation must be careful to avoid cultivating on a dry month.
D2, D3, D4	Only able to cultivate paddy once a year and production usually high due to high radiation density. These types have sufficient cultivation time for secondary crops.
E	Only able to cultivate of paddy or other secondary crops once a year, depend on the availability of irrigation water

Source: Alfiandy et al (2021)

**Table 4.** Climate shifting in Bali province

Location	Sub District	1991-2000	2001-2010	2011-2020	Trend
Kampung Anyar	Buleleng	D4	E4	E4	Tended to be dry
Sinabun	Sawan	D3	D4	E4	Drier
Depeha	Kubutambahan	D3	D4	D4	Tended to be dry
Pejarakan	Gerokgak	E4	E4	E4	Constant
Sembiran	Tejakula	D3	D4	D4	Tended to be dry
Banjarasem	Seririt	E4	E4	D3	Tended to be wet
Wanagiri	Sukasada	C3	C3	C3	Constant
Melaya	Melaya	E3	D3	E3	Varied
Berangbang	Negara	C3	D3	D3	Tended to be dry
Munduk	Banjar	C3	C3	C3	Constant
Candikuning	Baturiti	B3	C3	C3	Tended to be dry
Kintamani	Kintamani	D3	D3	D4	Tended to be dry
Pohsanten	Mendoyo	C3	D3	C3	Varied
Sepang	Busungbiu	B3	B3	C3	Tended to be dry
Pelaga	Petang	C3	C3	C3	Constant
Tulamben	Kubu	D3	D3	D4	Tended to be dry
Batungsel	Pupuan	B3	C2	B2	Varied
Pupuan	Tegallalang	C3	C3	C3	Constant
Pengotan	Bangli	B3	C2	D2	Drier
Besakih	Rendang	B3	C2	D2	Drier
Buanagiri	Bebandem	C3	D2	D2	Tended to be dry
Mangesta	Penebel	B2	B2	C2	Tended to be dry
Tua	Marga	B3	B3	C2	Tended to be dry
Buahan	Payangan	B3	B3	C2	Tended to be dry
Manukaya	Susut	B3	C2	C2	Tended to be dry
Yangapi	Tembuku	B3	C2	C2	Tended to be dry
Ababi	Abang	D3	D3	D3	Constant
Seraya Timur	Karangasem	E4	E3	D4	Wetter
Gumbrih	Pekutatan	C3	C2	D2	Varied
Mundeh	Selemadeg Barat	C3	C2	C2	Tended to be wet
Petiga	Marga	B3	B2	C2	Varied
Tegalalang	Tampaksiring	C3	B2	C2	Varied
Duda	Selat	B3	B1	D2	Varied
Berembeng	Selemadeg	C3	C2	C2	Tended to be wet
Meliling	Kerambitan	C3	C2	C3	Varied
Selanbawak	Marga	C3	C2	C2	Tended to be wet
Taman	Abiansemal	B2	C2	C2	Tended to be dry
Aan	Banjarangkan	C3	D1	D2	Drier
Kerthabuana	Sidemen	D3	D1	D1	Tended to be wet
Manggis	Manggis	E4	E3	D3	Wetter
Tegalmengkeb	Selemadeg Timur	C3	D3	D3	Tended to be dry
Delod Peken	Tabanan	C3	C2	C2	Tended to be wet
Sedang	Abiansemal	D3	D2	D3	Varied
Mas	Ubud	D3	D2	D3	Varied
Gianyar	Gianyar	E3	D1	D2	Varied
Tusan	Klungkung	E3	D1	E2	Varied
Gunaksa	Dawan	E3	D2	E2	Varied
Buwit	Kediri	C3	D3	D2	Varied
Sempidi	Mengwi	D3	D3	D3	Constant
Peguyangan Kangin	Denpasar Utara	D3	D3	D3	Constant
Sukawati	Sukawati	E3	D3	D3	Tended to be wet
Parerenan	Kuta Utara	C3	D3	D2	Varied
Kerobokan Kaja	Denpasar Barat	D3	D3	D3	Constant
Sumerta Kaja	Denpasar Timur	E3	D3	D3	Tended to be wet
Seminyak	Kuta	C3	D3	D4	Drier
Sidakarya	Denpasar Selatan	D3	D3	D4	Tended to be dry
Toyapakeh	Nusa Penida	E4	D4	D4	Tended to be wet
Jimbaran	Kuta Selatan	C3	D4	D4	Tended to be dry

through analysis of dry and wet months.

The highest suitability class for paddy was S2 (24 locations) with 41.38% in percentage, corn (S1) in 30 locations (51.72%), soybean (S1) in 29 locations (50.00%), shallot (S1) in 39 locations (67.24%), and chili (S2) in 28 locations (48.28%). Plants cultivated in suitable climate and

land conditions able to produce optimally, conversely plants in unsuitable climate and land conditions had an impact on decreasing production (Nganji and Simanjuntak 2020). Plants cultivated on land that very suitable to climatic conditions expected to produce higher levels of productivity than those cultivated on land that is quite suitable or

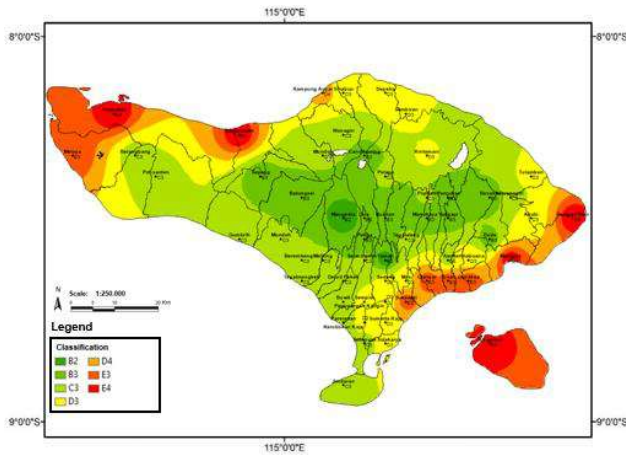


Fig.1. Agroclimatic Zone during 1991-2000

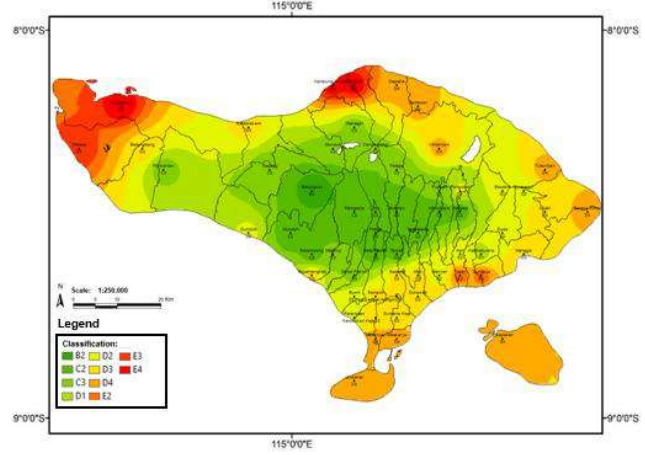


Fig. 3. Agroclimatic Zone during 2011-2020

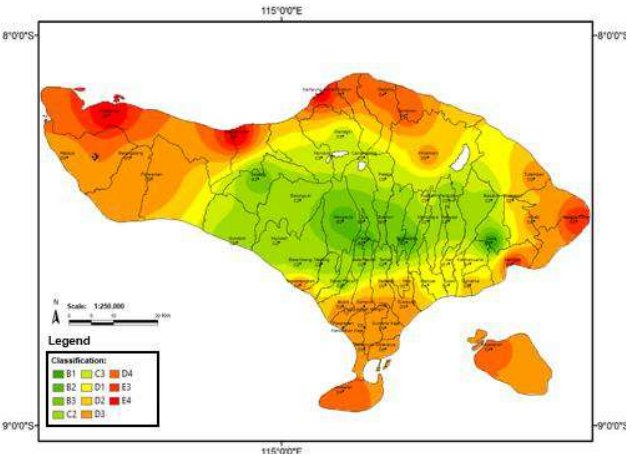


Fig. 2. Agroclimatic Zone during 2001-2010

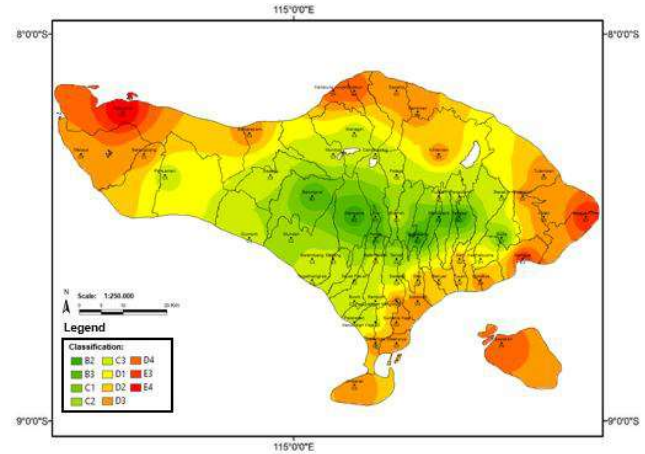


Fig. 4. Agroclimatic Zone during 1991-2020

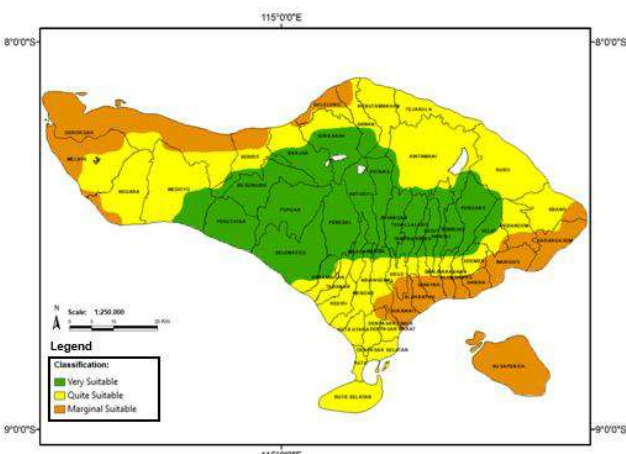


Fig. 5. Suitability for paddy

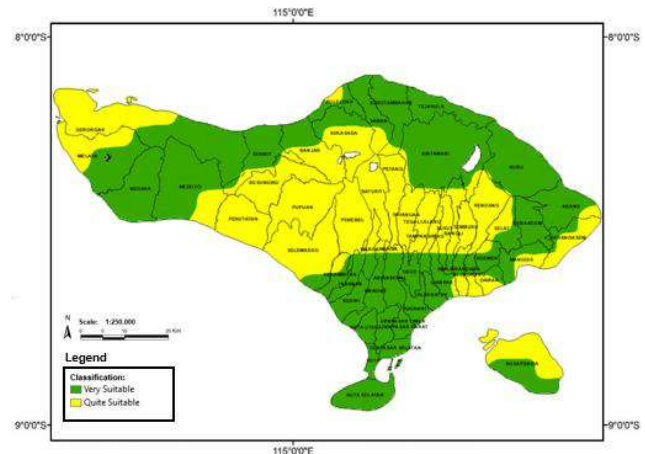


Fig. 6. Suitability for corn

**Table 5.** Recapitulation of agroclimatic land suitability for paddy, corn, soybean, shallot and chili

Grid/ Location	Sub district	Climate types	Land suitability				
			Paddy	Corn	Soybean	Shallot	Chili
Kampung Anyar	Buleleng	D4	S3	S2	S1	S2	S3
Sinabun	Sawan	D4	S3	S1	S1	S2	S2
Depeha	Kubutambahan	D3	S2	S1	S1	S2	S2
Pejarakan	Gerokgak	E4	S3	S2	S1	S2	S3
Sembiran	Tejakula	D3	S2	S1	S1	S2	S2
Banjarasem	Seririt	D3	S3	S1	S1	S2	S2
Wanagiri	Sukasada	C3	S1	S2	S2	S1	S1
Melaya	Melaya	D3	S3	S2	S1	S1	S3
Berangbang	Negara	D3	S2	S1	S1	S1	S2
Munduk	Banjar	C3	S1	S2	S2	S1	S1
Candikuning	Baturiti	C3	S1	S2	S2	S1	S1
Kintamani	Kintamani	D3	S2	S1	S1	S1	S2
Pohsanten	Mendoyo	C3	S2	S1	S2	S1	S1
Sepang	Busungbiu	C3	S1	S2	S2	S1	S1
Pelaga	Petang	C3	S1	S2	S2	S1	S1
Tulamben	Kubu	D3	S2	S1	S1	S1	S2
Batungsel	Pupuan	B3	S1	S2	S3	S1	S2
Pupuan	Tegalalang	C3	S1	S2	S2	S1	S1
Pengotan	Kintamani	C3	S1	S2	S2	S1	S1
Besakih	Rendang	C3	S1	S2	S2	S1	S1
Buanagiri	Bebandem	D3	S2	S1	S1	S1	S2
Mangesta	Penebel	B2	S1	S2	S3	S3	S2
Tua	Marga	B3	S1	S2	S3	S1	S2
Buahan	Petang	C3	S1	S2	S2	S1	S1
Manukaya	Susut	B3	S1	S2	S3	S1	S2
Yangapi	Tembuku	B2	S1	S2	S3	S3	S2
Ababi	Abang	D3	S2	S1	S1	S1	S2
Seraya Timur	Karangasem	E3	S3	S2	S1	S2	S3
Gumbrih	Pekutatan	C3	S1	S2	S2	S1	S1
Mundeh	Selemadeg Barat	C2	S1	S2	S2	S3	S1
Petiga	Marga	B3	S1	S2	S3	S1	S2
Tegalalang	Tampaksiring	B2	S1	S2	S3	S3	S2
Duda	Selat	C1	S2	S1	S2	N	S1
Berembeng	Selemadeg	C3	S1	S2	S2	S1	S1
Meliling	Kerambitan	C3	S1	S2	S2	S1	S1
Selanbawak	Marga	C3	S2	S1	S2	S1	S1
Taman	Abiansemal	C2	S2	S1	S2	S3	S1
Aan	Banjarangkan	D2	S2	S1	S1	S3	S2
Kerthabuana	Sidemen	D1	S2	S1	S1	N	S2
Manggis	Manggis	E3	S3	S2	S1	S1	S3
Tegalmengkeb	Selemadeg Timur	C3	S2	S1	S2	S1	S1
Delod Peken	Tabanan	C3	S2	S1	S2	S1	S1
Sedang	Abiansemal	C3	S2	S1	S1	S1	S2
Mas	Ubud	D2	S3	S1	S1	S3	S2
Gianyar	Gianyar	D2	S3	S1	S1	S3	S2
Tusan	Klungkung	D2	S3	S2	S1	S3	S3
Gunaksa	Dawan	D3	S3	S2	S1	S1	S3
Buwit	Kediri	C3	S2	S1	S2	S1	S1
Sempidi	Mengwi	C3	S2	S1	S2	S1	S1
Peguyangan Kangin	Denpasar Utara	D3	S3	S1	S1	S1	S2
Sukawati	Sukawati	D3	S3	S1	S1	S1	S2
Parerenan	Kuta Utara	C3	S2	S1	S2	S1	S1
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Sumerta Kaja	Denpasar Timur	D3	S2	S1	S1	S1	S2
Seminyak	Kuta Utara	D3	S2	S1	S1	S1	S2
Sidakarya	Denpasar Selatan	D3	S2	S1	S1	S1	S2
Toyapakeh	Nusa Penida	D4	S3	S2	S1	S2	S3
Jimbaran	Kuta Selatan	D3	S2	S1	S1	S1	S2



marginally suitable. The next stage is to create land suitability class map for these commodities.

The suitability class for paddy ranged between very suitable to marginally suitable. Land with very suitable classes located in the central part has higher precipitation

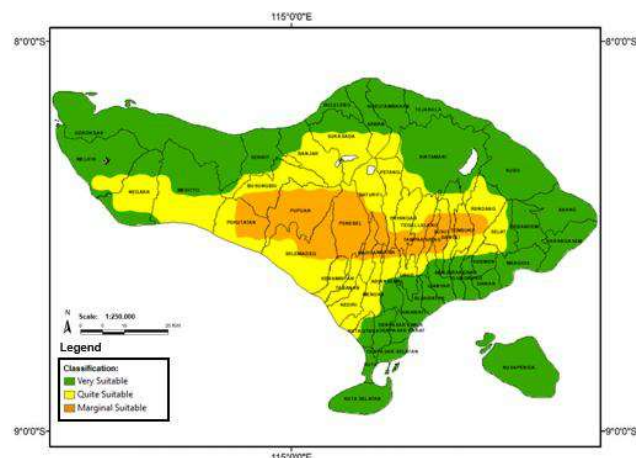


Fig. 7. Suitability for soybean

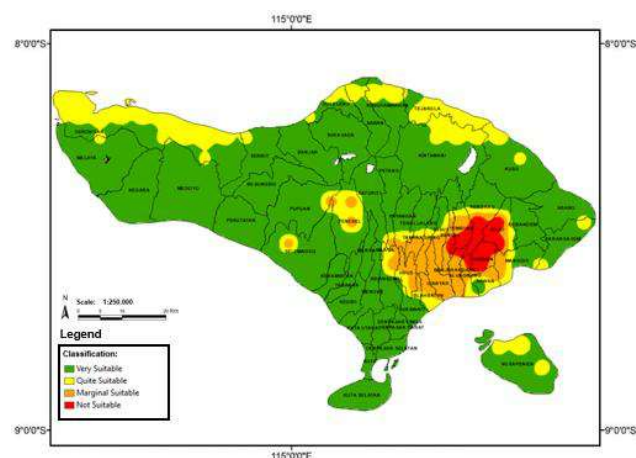


Fig. 8. Suitability for shallot

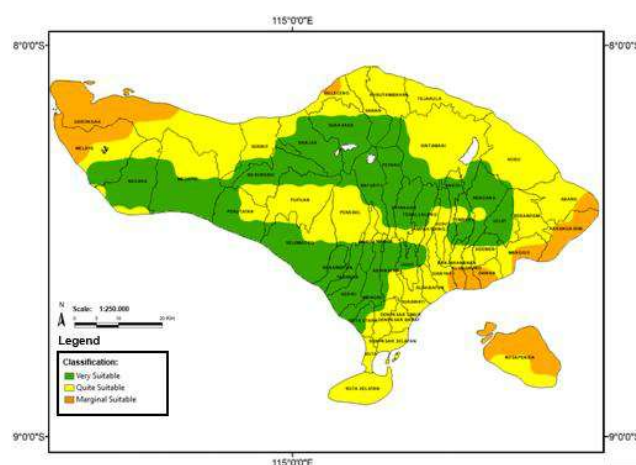


Fig. 9. Suitability for chili

because it is influenced by orographic rain. Meanwhile in western and southeast parts considered as areas of less in wet months. Land suitability classification for corn commodities is very suitable or have a number of wet months in a row between 3-5 months per year covering the outermost land except the western, central and eastern tip and the southern Nusa Penida.

Soybean can be well cultivated in areas of D1 – D4 in types, and at 0-500 meters of altitude above mean sea level. Areas with suitability class S1 distributed in outermost area except the southwest and central parts. By knowing this map, farmers will be able to plan the way to increase soybean production again in areas of S1 and S2 suitability classes.

Suitability for shallot ranges from very suitable to not suitable. Some areas that are not suitable for shallot cultivation because the number of dry months less than two months and have Oldeman's climate types other than C1 – C4. Suitability for chili ranges from very suitable to marginally suitable. It is hoped that these maps will help farmers in Bali to determine the precious time to start cultivating. Bali has experienced a decline in chili production over the last three years (2022-2024). By knowing above suitability map, it will help farmers to increase production of chili production again.

## CONCLUSION

Central part of Bali considered as areas with more wet months, while the outermost parts except the southwest tend to have fewer wet months. This condition causes agricultural commodities that depend on wet months to be more suitable if cultivated there, taking into account the suitability of crops for height. The climate types shifting during 1991-2020 period showed that 49 (84.48%) locations experienced shifting, while 9 (15.52%) other locations did not experience shifting or remained the same. Research using longer data such as 30-year data able to provide a more complete picture of shift patterns than shorter data such as 10-year data. The author suggests climate types of 1991-2020 period to be used in agricultural planning.

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Received 22 September, 2024; Accepted 24 January, 2025



# Variation for Qualitative Leaf Morphometric Traits among Half-Sib Progenies of *Cinnamomum zeylanicum* Blume. in the Western Ghats (Hill zone), Karnataka

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**Abstract:** *Cinnamomum zeylanicum* (Lauraceae) known as 'true cinnamon' or 'sweet wood', native species of Sri Lanka and the Indian West Coast, is commercially valuable tree spice in India. Cinnamon leaves are widely used as spice, and possess an essential oil rich in eugenol, which is a highly sought after by the perfume and flavour industries. The study was undertaken during 2018-2020 at College of Forestry, Sirsi, Uttara Kannada. Seeds were collected from 106 superior mother trees of five different sources during June-July and progenies were established in nursery. After 24 months, qualitative leaf traits of progenies were evaluated. Wide variation was observed with respect to leaf parameters among progenies of different sources and even some progenies exhibited variation from its mother plants. Leaf colour, leaf petiole colour and leaf margin serration is almost similar in all the progenies; progenies of tree G2 and K2 recorded light purple and purple colour petiole, respectively as this character is entirely different from the entire progenies. The majority of progenies exhibited light purple colour leaves (50.94%) followed by medium purple colour leaves (28.30%). Only two progenies, G2 and K2 recorded the purple colour petiole with sweet taste. Five types of leaf shapes (elliptic, oblong, ovate, ovate elliptic and ovate lanceolate) and four leaf tip shapes (acuminate, obtuse, acute and sub acute) were recorded among selected progenies. G2 progeny produced oblong shaped leaves and majority of the progenies recorded elliptic leaf shape (51.89%) and sub acute leaf tip shape (69.81%). There was no variation among the progenies with respect to leaf margin (all are entire type). Leaf markers that could be adopted easily to measure the magnitude of diversity, to select higher yielding types as well as for conservation.

**Keywords:** Western Ghats, Cinnamon, Qualitative parameter, Leaf flush colour

*Cinnamomum zeylanicum* (Lauraceae) popularly known as 'true cinnamon/cinnamon' is a native of Sri Lanka and the West coast region of India (Ravindran et al., 2004). It is believed that the genus *Cinnamomum* has a centre of diversity in the Western Ghats and the adjoining regions of South India (Sasikumar et al., 1999). Wide variability of the *Cinnamomum* species occurs in the Western Ghats and in some parts of North Eastern states of India. Of all the characters, qualitative and quantitative leaf traits are highly variable in the genus *Cinnamomum* and this variation is seen both at species and sub species levels. (Ravindran et al., 2004, Hanumantha 2020, Hanumantha et al., 2020). Leaf characteristics are highly variable in the genus *Cinnamomum* and these variation in leaf parameters can be used to recognize and describe species level similarities Niharika and Hanumantha (2024).

The flushing time coincides with the monsoon. Four different flush colours are noted among the cinnamon collections, viz., pure purple, purple dominated with green, green dominated with purple and pure green. Considering parameters such as bark pungency, leaf morphology, grittiness of the bark and leaves, eight types of cinnamon are recognized by cinnamon growers in Sri Lanka

(Ravindran et al., 2004). Morphological characters are markers that are adopted to measure the magnitude of diversity in plants based on the phenotype character (Lizawati et al., 2018). Interactions of genotype and environmental factors play a role in generating such wide variations when planted in varied environments (De Leon et al., 2016). These variations could be adopted in selection of higher yielding types when the traits are genetically correlated. For instance, Wijesinghe and Gunarathna (2001) has shown that there is a positive correlation between leaf size and shape with yield in seven different types of cinnamon and reported that with large round leaves had high bark yield; high cinnamaldehyde content in inwardly curved leaves and high quality oil from the small round leaves.

Wide variability of cinnamon is also present in farmers' fields of Karnataka in terms of leaf traits because most of the farmer's plant trees originating from un-tested and un-domesticated sources (Hanumantha et al., 2020). Therefore, it is necessary to assess the variation present in leaf morphometric traits among half sib progenies of cinnamon, collected and raised from different superior trees of the Central Western Ghats.

## MATERIAL AND METHODS

The present study was undertaken during 2018-2020 at the College of Forestry, Sirsi, and at various field sites of hill zone of Karnataka. Uttara Kannada district located in the Central Western Ghats between 13° 55' to 15° 32' N latitude and 74° 05' to 75° 05' E longitude with a geographic area of 10,291 km<sup>2</sup>. In this study five plantation areas in three districts of the Karnataka namely Uttara Kannada (Jaddigadde, Kankodlu and Siddapura), Shivamogga (Manchale) and Haveri (Gejjehalli) (Table 1). Superior trees in each plantation were selected based on eye ball screening and based on the

experience of the plantation owners. Totally 106 mother trees were selected from five even aged plantations (Table 1). Seeds were collected from the selected 106 mother trees and progenies were established. After 24 months qualitative leaf parameters were recorded for the progenies and data was used for further tabulation.

## RESULTS AND DISCUSSION

**Variation in qualitative leaf parameters among half sib progenies:** Wide variation was observed with respect to leaf parameters among progenies of different sources and even some progenies exhibited variation from its mother plants. Leaf colour, leaf petiole colour and leaf margin serration is almost similar in all the progenies; progenies of tree number G2 and K2 recorded light purple and purple colour petiole respectively as this character is entirely different from the entire progenies (Table 4, 6). The leaf colour and leaf margin serration is almost similar in the entire progenies and noticed dark green colour leaves and entire leaf margin serration (Table 4-8). In leaf colour, from Jaddigadde three progenies

**Table 1.** Details of mother tree sources with their IDs

Place of collection	Taluk	Tree IDs	Number superior trees selected
Gejjehalli	Hangal	G1 to G25	25
Jaddigadde	Sirsi	J1 to J25	25
Kankodlu	Yellapur	K1 to K25	25
Manchale	Sagara	M1 to M21	21
Siddapura	Siddapura	S1 to S10	10
Total			106

**Table 2.** Brief review of the works carried out on leaf parameters of cinnamon trees/progenies

Leaf parameter	Procedure/descriptors used	Reference
Leaf flush colour	Green, Light pink / purple, Medium pink / purple, Deep pink / purple, Very deep pink/purple	Krishnamoorthy et al (1988) and (1992), Joy et al (1998)
Petiole colour	Green, Light purple, Purple	--
Leaf colour	Pale / light green, Green, Dark Green	Joy et al (1998), Azad et al (2016), Lizawatiet al (2018)
Leaf shape	Elliptic, Ovate, Ovate-elliptic Ovate-lanceolate Oblong	
Leaf tip/apex shape	Obtuse, Sub-acute, Acuminate, Acute	
Leaf margin serration	Entire and Wavy	
Leaf parameter variations for mother trees (Plantation trees)	Variation for leaf flush colour, leaf colour, leaf tip shape, leaf margin, leaf shape, petiole colour among different sources and mother trees	Hanumantha et al (2020)
Leaf parameter variations among mother trees (Natural trees)	Variation for leaf flush colour, leaf colour, leaf tip shape, leaf margin, leaf shape and petiole colour, among different sources and mother trees	Sourav Manoharan and Hanumantha (2023)

**Table 3.** Geo-locations and characteristic forest types of the seed sources of progenies considered in the study

Seed source	District, nearest forest type, population size of Cinnamon trees	Latitude & longitude	Altitude (m)	Number of mother trees selected	No. of progenies raised from each source	No. of progenies raised and evaluated per mother tree
Gajjehalli	Haveri Scrub forest (n=200)	N 14°44'14.9" E 75°07'56.6"	584 m	25	25	30
Jaddigadde	Uttara Kannada Semi-evergreen forest (n=200)	N 14°48'09.2" E 74°44'32.9"	486 m	25	15	30
Kankodlu	Uttara Kannada Evergreen forest (n= 450)	N 14°45'10.9" E 74°50'53.9"	474 m	25	25	30
Manchale	Shivamogga Semi-evergreen forest (n=450)	N 14°10'21.9" E 75°05'57.1"	624 m	21	15	30
Siddapura	Uttara Kannada Evergreen forest (n=100)	N 14°20'14.8" E 74°52'35.6"	584 m	10	10	30
Total					90	

(J1, J8 and J24), Kankodlu two progenies (K1 and K23) and from Manchale five progenies (M1, M12, M14, M17 and M18) recorded green coloured leaves. Leaf flush colour among progenies of different sources varied from green colour to pink colour; only three progenies G25, J4 and K2 recorded purple colour and remaining all progenies recorded from green to medium pink flush colour. With respect to leaf shape, the progenies recorded ovate, elliptic, ovate lanceolate, ovate elliptic shaped leaves; but interestingly G2 showed oblong shaped leaves (Table 4). Leaf tip also varied among progenies; majority of the progeny leaves recorded sub-acute tip shape followed by acuminate leaf tip shape; but tree number M1 showed obtuse leaf tip shape.

Among all 90 progenies (Fig. 1), majority of progenies exhibited light purple colour leaves (50.94%) followed by medium purple colour leaves (28.30%), only 3.78 per cent of

the progenies recorded purple colour leaf flush and remaining progenies recorded green flush colour (16.98%). With respect to mature leaf colour (Fig. 2) majority of progenies produced dark green colour leaves (85.85%) followed by green colour leaves (14.15%). Only little variation was observed among the progenies for leaf petiole colour; only two progenies differed for petiole colour. Progeny of K2 (Kankodlu) exhibited purple colour petiole followed by progeny of G2 (Gejjehalli) light purple colour petiole and remaining 88 progenies recorded green colour petiole. Variation with respect to leaf shape and leaf tip shape also recorded among selected progenies (Fig. 3, 4). Five types of leaf shapes (elliptic, oblong, ovate, ovate elliptic and ovate lanceolate) and four leaf tip shapes (acuminate, obtuse, acute and sub acute) were recorded among selected progenies. Among all the progenies only G2 progeny produced oblong

**Table 4.** Variation for qualitative leaf characteristics among progenies of *C. zeylanicum* (Gejjehalli)

Progeny No.	Leaf flush colour	Leaf colour	Leaf shape	Leaf tip shape
G1	2	3	Ovate	Sub-Acute
G2	3	3	Oblong	Sub-Acute
G3	2	3	Ovate-Lanceolate	Sub-Acute
G4	2	3	Ovate-Lanceolate	Acuminate
G5	3	2	Ovate	Acuminate
G6	2	3	Elliptic	Sub-Acute
G7	2	3	Ovate-Lanceolate	Sub-Acute
G8	2	3	Elliptic	Sub-Acute
G9	1	3	Ovate-Lanceolate	Acuminate
G10	3	2	Elliptic	Acuminate
G11	3	3	Ovate	Sub-Acute
G12	1	3	Elliptic	Sub-Acute
G13	3	3	Elliptic	Sub-Acute
G14	2	3	Ovate	Sub-Acute
G15	2	3	Elliptic	Acuminate
G16	3	3	Elliptic	Sub-Acute
G17	1	3	Ovate	Sub-Acute
G18	2	3	Elliptic	Sub-Acute
G19	2	3	Ovate	Sub-Acute
G20	1	3	Elliptic	Sub-Acute
G21	2	3	Elliptic	Sub-Acute
G22	1	3	Elliptic	Sub-Acute
G23	2	3	Ovate	Sub-Acute
G24	3	3	Elliptic	Sub-Acute
G25	4	3	Elliptic	Sub-Acute

\* All the progenies exhibited green petiole colour; but G2 exhibited light purple colour

\*\*All the progenies exhibited entire leaf margin serration

Leaf flush colour: 1= Green 2= Light purple 3= Medium purple 4= Purple 5= Dark purple

Leaf colour: 1=Light green 2= Green 3= Dark green

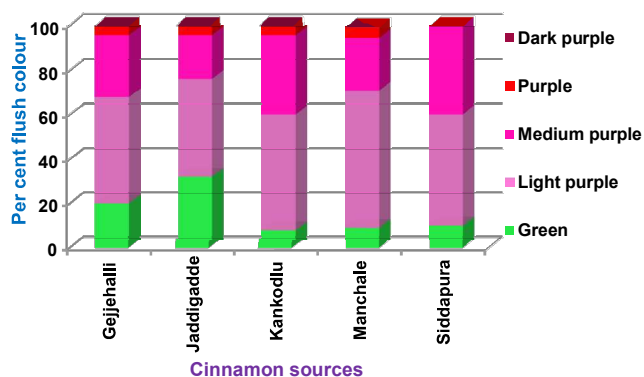


Fig. 1. Variation in leaf flush colour among progenies of different sources

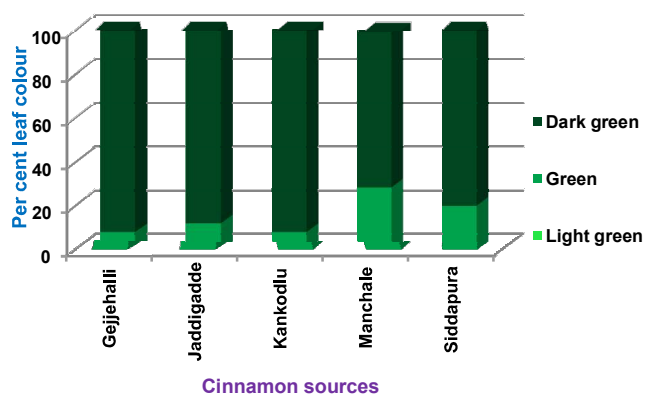


Fig. 2. Variation in leaf colour among progenies of different sources

Table 5. Variation for qualitative leaf characteristics among progenies of *C. zeylanicum* (Jaddigadde)

Progeny No.	Leaf flush colour	Leaf colour	Leaf shape	Leaf tip shape
J1	2.0	2.0	Ovate	Acuminate
J2	3.0	3.0	Ovate	Acuminate
J3	2.0	3.0	Ovate	Sub-Acute
J4	4.0	3.0	Elliptic	Sub-Acute
J5	1.0	3.0	Elliptic	Acuminate
J6	2.0	3.0	Elliptic	Sub-Acute
J7	3.0	3.0	Ovate	Acuminate
J8	1.0	2.0	Elliptic	Acuminate
J9	2.0	3.0	Elliptic	Sub-Acute
J10	1.0	3.0	Elliptic	Acuminate
J11	3.0	3.0	Ovate	Sub-Acute
J12	2.0	3.0	Elliptic	Sub-Acute
J13	2.0	3.0	Elliptic	Acuminate
J14	2.0	3.0	Elliptic	Sub-Acute
J15	1.0	3.0	Ovate-Elliptic	Sub-Acute
J16	1.0	3.0	Elliptic	Sub-Acute
J17	2.0	3.0	Ovate	Sub-Acute
J18	3.0	3.0	Elliptic	Sub-Acute
J19	1.0	3.0	Elliptic	Acuminate
J20	2.0	3.0	Elliptic	Sub-Acute
J21	2.0	3.0	Ovate	Sub-Acute
J22	2.0	3.0	Elliptic	Sub-Acute
J23	1.0	3.0	Ovate-Elliptic	Acuminate
J24	1.0	2.0	Elliptic	Sub-Acute
J25	3.0	3.0	Elliptic	Sub-Acute

\*All the progenies exhibited green petiole colour

\*\* All the progenies exhibited entire leaf margin serration

\*\*\* See the Table 2 for details of leaf flush colour and leaf colour

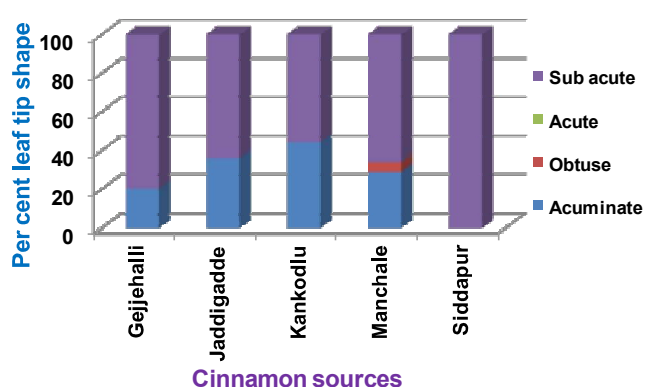
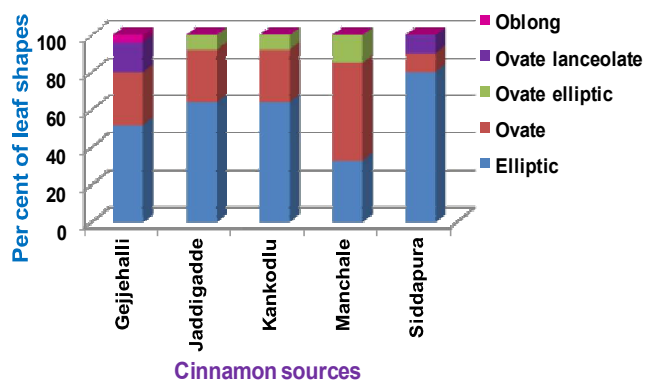


Fig. 3. Variation in leaf shape among progenies of different sources

Fig. 4. Variation in leaf tip shape among progenies of different sources

Table 6. Variation for qualitative leaf characteristics among progenies of *C. zeylanicum* (Kankodlu)

Tree No.	Leaf flush colour	Leaf colour	Leaf shape	Leaf tip shape
K1	2	2	Ovate	Sub-Acute
K2	4	3	Ovate	Sub-Acute
K3	3	3	Ovate	Sub-Acute
K4	3	3	Elliptic	Acuminate
K5	2	3	Elliptic	Sub-Acute
K6	3	3	Elliptic	Acuminate
K7	3	3	Elliptic	Acuminate
K8	1	3	Elliptic	Acuminate
K9	3	3	Elliptic	Sub-Acute
K10	1	3	Elliptic	Sub-Acute
K11	2	3	Ovate	Sub-Acute
K12	2	3	Ovate	Sub-Acute
K13	3	3	Elliptic	Sub-Acute
K14	3	3	Elliptic	Acuminate
K15	2	3	Elliptic	Sub-Acute
K16	3	3	Ovate-Lanceolate	Sub-Acute
K17	2	3	Ovate	Acuminate
K18	2	3	Elliptic	Acuminate
K19	3	3	Ovate	Sub-Acute
K20	2	3	Ovate-Lanceolate	Acuminate
K21	2	3	Ovate-Lanceolate	Sub-Acute
K22	2	3	Ovate	Acuminate
K23	2	2	Ovate	Acuminate
K24	2	3	Ovate	Sub-Acute
K25	2	3	Ovate	Acuminate

\* All the progenies exhibited green petiole colour; but K2 exhibited light purple colour

\*\*All the progenies exhibited entire leaf margin serration

\*\*\* See the Table 2 for details of leaf flush colour and leaf colour

shaped leaves. Majority of the progenies recorded elliptic leaf shape (51.89%) and sub acute leaf tip shape (69.81%) leaves. There was no variation among the progenies with respect to leaf margin (all are entire margin type).

**Characterization of qualitative traits of half sib progenies** : Wide variation was observed with respect to leaf parameters among progenies of different sources and

some progenies exhibited variation from its mother plants. Leaf flush colour among progenies of different sources varied from green colour to pink colour (Fig. 1). Only three progenies G25, J4 and K2 recorded purple colour and remaining all progenies exhibited green to medium pink flush colour (Plate 1a). The progenies recorded ovate, elliptic, ovate lanceolate, ovate elliptic shaped leaves; but

**Table 7.** Variation for qualitative leaf characteristics among progenies of *C. zeylanicum* (Manchale)

Progeny No.	Leaf flush colour	Leaf colour	Leaf shape	Leaf tip shape
M1	2.0	2.0	Elliptic	Obtuse
M2	2.0	3.0	Elliptic	Sub-Acute
M3	2.0	3.0	Ovate-Elliptic	Acuminate
M4	2.0	3.0	Ovate	Sub-Acute
M5	3.0	3.0	Ovate-Elliptic	Sub-Acute
M6	2.0	3.0	Ovate	Sub-Acute
M7	2.0	3.0	Elliptic	Sub-Acute
M8	2.0	3.0	Ovate	Sub-Acute
M9	2.0	2.0	Ovate	Sub-Acute
M10	3.0	3.0	Ovate	Acuminate
M11	2.0	3.0	Ovate	Sub-Acute
M12	3.0	2.0	Elliptic	Acuminate
M13	2.0	3.0	Ovate	Sub-Acute
M14	2.0	2.0	Ovate	Acuminate
M15	1.0	3.0	Elliptic	Sub-Acute
M16	3.0	3.0	Ovate-Elliptic	Sub-Acute
M17	1.0	2.0	Ovate	Acuminate
M18	2.0	2.0	Elliptic	Acuminate
M19	4.0	3.0	Ovate	Sub-Acute
M20	2.0	3.0	Ovate	Sub-Acute
M21	3.0	3.0	Elliptic	Sub-Acute
Mean	2.20	2.70		
S.D	0.70	0.46		
C.V (%)	31.29	17.05		

\*All the progenies exhibited green petiole colour; \*\* All the progenies exhibited entire leaf margin serration

\*\*\* See the Table 4 for details of leaf flush colour and leaf colour

**Table 8.** Variation for qualitative leaf characteristics among progenies of *C. zeylanicum* (Siddapura)

Progeny No.	Leaf flush colour	Leaf colour	Leaf shape	Leaf tip shape
S1	3.0	3.0	Elliptic	Sub-Acute
S2	2.0	3.0	Elliptic	Sub-Acute
S3	2.0	2.0	Elliptic	Sub-Acute
S4	3.0	3.0	Elliptic	Sub-Acute
S5	2.0	3.0	Elliptic	Sub-Acute
S6	2.0	3.0	Elliptic	Sub-Acute
S7	2.0	2.0	Ovate-Lanceolate	Sub-Acute
S8	1.0	3.0	Ovate	Sub-Acute
S9	3.0	3.0	Elliptic	Sub-Acute
S10	3.0	3.0	Elliptic	Sub-Acute

\*All the progenies exhibited green petiole colour; \*\* All the progenies exhibited entire leaf margin serration

\*\*\* See the Table 4 for details of leaf flush colour and leaf colour





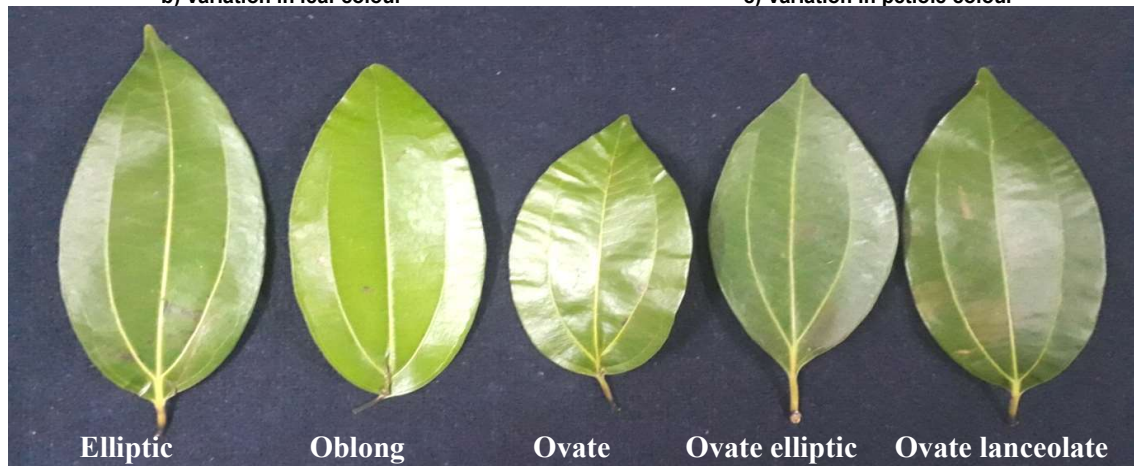
a) Variation in leaf flush colour



b) Variation in leaf colour



c) Variation in petiole colour



d) Variation in leaf shape



e) Variation leaf tip shape

Plate 1. Variation in leaf morphology among selected progenies of *C. zeylanicum*

interestingly G2 showed oblong shaped leaves (Plate 1d). Leaf tip also varied among progenies; majority of the progeny leaves recorded sub-acute leaf tip shape followed by acuminate leaf tip shape; but progeny of tree M1 showed obtuse leaf tip shape (Plate 1e). Over all, among 90 progenies, majority of trees exhibited light purple colour leaf flush (50.94%) followed by medium purple colour leaf flush (28.30%), 3.78 per cent of the progenies recorded purple colour leaf flush and 16.98 per cent green flush colour. In mature leaf colour (Fig 1, Plate b), majority of progenies produced dark green colour leaves (85.85%) followed by green colour leaves (14.15%). Little variation was observed among progenies for leaf petiole colour; only two progenies differed for petiole colour. Progeny of K2 (Kankodlu) exhibited purple colour petiole followed by progeny of G2 (Gejjehalli) light purple colour petiole and remaining progenies recorded green colour petiole (Plate 1c). Variation with respect to leaf shape and leaf tip shape also recorded among the progenies (Fig. 3, 4). Among all the progenies only G2 progeny produced oblong shape leaves. Majority of the progenies recorded elliptic shape leaves (51.89%) followed by ovate (34.91%) and for leaf tip shape sub-acute tip shape (69.81%) is dominated followed by acuminate type (29.25%). All the progenies leaves showed entire margin type. Several authors reported variations in leaf flush colour, leaf colour, leaf shape, leaf tip shape and leaf margin as reported in mother trees.

Variation between mother trees and progenies also observed for leaf parameters. Majority of the progenies expressed sub-acute type leaf tips as compared to mother trees. In progenies oblong leaf shape is recorded only for one progeny (G2). Azad et al (2015) reported variation in leaf characteristics (leaf shape, leaf base and leaf apex) of mother trees and progenies. Having different alleles in population, cross pollination can help different allelic combinations in the progeny. Such different allelic combinations can lead to new phenotypes in the progeny. Hanumantha et al (2020) reported elliptic, ovate, ovate-elliptic and ovate lanceolate leaf shape and sub-acute leaf tip shape followed by obtuse and acuminate among the majority of selected trees in *Cinnamomum zeylanicum*. Sourav Manoharan and Hanumantha (2023) reported five types of leaf shapes viz., elliptic, oblong, ovate, ovate-elliptic and ovate lanceolate and four types of leaf apex viz., acuminate, acute, sub-acute and obtuse among trees of six different sources in Uttara Kannada, Karnataka. Elliptic leaf shape and acuminate leaf tip was predominantly expressed and suggested that leaf morphometric traits could be easily used to measure the magnitude of diversity. Dattappa et al (2023) reported variation among 15 half sib

progenies of cinnamon for leaf weight, leaf area and leaf weight per plant.

Variation in petiole colour was recorded for the first time in cinnamon and two progenies namely G2 and K2 exhibited purple coloured petioles. Even the taste and pungency of the petiole is entirely different from the other progenies; they have sweeter taste and lesser pungency. This character can be utilized for differentiating the individuals of cinnamon for future selection purposes. Cinnamon develops wide variety of flush colours and ranges from green to deep purple. Significant positive correlation between leaf flush colour and leaf oil content was observed suggesting genotypes with dark purple colour flush tend to possess higher in the leaf oil content. Hence, the leaf flush colour can be used as important qualitative parameter for selection of trees with high oil content. The quick visual observation of purple leaf flush colour will be useful for selection for higher leaf oil yield at least in preliminary screening. The study demonstrated that traits such as leaf colour, flush colour, leaf margin, leaf shape and leaf petiole traits could be considered while developing Distinctness, Uniformity and Stability (DUS) traits as descriptors.

## CONCLUSION

*Cinnamomum zeylanicum* is one of the most valuable tree spices in Karnataka. Wide variability is present among the different species of cinnamon. The variations present among the different source can be used for identification of good genotypes based on the results of progeny tests. Wide variation was observed for leaf characteristics among progenies of different sources in *Cinnamomum zeylanicum*. Five morpho-types with respect to leaf shapes viz. elliptic, oblong, ovate, ovate elliptic and ovate lanceolate were recorded, in which elliptic type of leaf shape was predominant. With respect to leaf tip shapes, four types viz., acuminate, obtuse, acute and sub-acute types were recorded among which, sub-acute tip shape was predominant. Only two trees (K2 from Kankodlu and G2 from Gejjehalli source) showed purplish colouration; all other trees showed green petiole colour. Leaf flush colour is considered as indicator of higher essential oil and can be used as marker for indirect selection of higher oil yielding trees. Leaf markers that could be adopted to easily measure the magnitude of diversity, to select higher yielding types as well as for conservation.

## ACKNOWLEDGEMENT

The authors are grateful to Dr. Srikanth Gunaga, Taxonomist, who helped in characterization of leaf parameters.

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# Distribution Pattern and Phytosociological Attributes of Understory Vegetation in Different Agroforestry Systems of Kashmir, India

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**Abstract:** The investigation was carried out in seven different silvipastoral systems viz *Cedrus deodara*, *Robinia pseudoacacia*, *Cupressus torulosa*, *Prunus armeniaca*, *Ailanthus altissima*, mixed plantations and grassland (control). There were 12 shrub and 43 herb species in all the systems. Among shrubs, *Berberis lycium*, and among herbs, *Cyanodon dactylon* were observed in all the systems. Maximum height (23.91 m) and maximum dbh (91.72 cm) was for the trees in *Robinia pseudoacacia* and *Cupressus torulosa* system, respectively. The highest density (8133 shrubs/ha) was in grassland (control) followed by *Prunus armeniaca*>*Robinia pseudoacacia*>mixed plantations>*Cupressus torulosa*. The highest density for *Cyanodon dactylon* (86666 tillers/ha) and lowest (10000 tillers/ha) was recorded for *Stipa sibirica*. The basal area (m<sup>2</sup>ha<sup>-1</sup>) of herbage in different treatments showed the precedence grassland (control) followed by *Prunus armeniaca*>*Robinia pseudoacacia*>*Ailanthus altissima*. Importance value index of different herbage species in different silvipastoral systems revealed that *Cyanodon dactylon* was the dominating species under grassland (control), *Ailanthus altissima* and mixed plantations system. *Oxalis acetosella* was dominating under *Cedrus deodara*. *Lolium perenne* was dominating species under mixed plantations, *Robinia pseudoacacia* and *Prunus armeniaca* agroforestry systems.

**Keywords:** Plantation, Density, Herbs, Grassland, Shrubs, Understory vegetation, Agroforestry systems

Botanical evaluations, encompassing examinations of floristic composition, species diversity, and structural analysis, are fundamental for gaining a comprehensive understanding of forest ecology and ecosystem functions (Pappoe et al., 2010). The definition of floristic composition, which signifies the variety of species within a community, necessitates precise identification of the species present. The determination of floristic composition is an intricate, prolonged process affected by seasonal fluctuations, biotic factors, microclimate, and their interactions within the community.

Individuals belonging to various species in a plant community assume distinct roles in ecosystem functioning, influencing diverse distribution patterns. To comprehend community structure, an examination of the spatial distribution of individuals from each species, floristic composition and distribution of plant communities under different traditional agroforestry systems in Takoli Gad watershed of Garhwal himalayas (Thakur et al., 2005, Bhusara et al., 2016, Singh et al., 2023). Community diversity, portraying variations in species and their quantitative attributes, is gauged to evaluate associations with community properties or environmental conditions In grassland communities, trees are pivotal, displaying varying density and types across diverse locations (Thakur et al.,

2004). India's diverse climate supports 15 types of native woodlands, where trees play a pivotal role in preserving moisture, enhancing soil quality, and offering nutritious feed for animals. Trees significantly contribute to landscape biomass and diversity, with a well-established pivotal role in ecosystem dynamics. Despite prior research on phytosociological attributes, information on the remote areas of Sindh Forest Division, Ganderbal Kashmir, is limited. This research initiative aims to establish a baseline for forthcoming studies, particularly crucial for monitoring plantation areas rich in biological diversity. No sincere effort has been made in this area to analyse the community structure of understory vegetation under different agroforestry systems over time in the past. Keeping in view this fact the study was conducted in Sindh forest division Ganderbal in different agroforestry systems to compare the floristic composition and community structure of these systems with grassland (control) which was devoid of trees.

## MATERIAL AND METHODS

The even aged mature stands of different kinds of trees were selected for study and evaluated the community structure of different agroforestry systems. The seven land use systems selected were T1-(*Cedrus deodara*), T2-(*Robinia pseudoacacia*), T3-(*Cupressus torulosa*), T4-

(*Prunus arminiaca*), T5-(*Ailanthus altissima*), T6-Mixed stand (*Cupressus torulosa*, *Robinia pseudoacacia*, *Ailanthus altissima*), T7-Grassland (No trees).

**Shrub density:** The number of shrubs under each agroforestry systems was done by stratified random sampling in 3 quadrates of size 5×5 m in each sampling plot. The total number of shrubs of each species were counted in each quadrate and categorized into large, medium and small shrubs.

**Herb density:** Phytosociological attributes of herb species under different agroforestry systems were evaluated from 3 quadrates of size 1×1 m in each sampling plot. The samples collected were brought to the laboratory, washed properly with fresh running water and segregated species-wise. The individuals of each species from different quadrates were counted separately and their basal area was calculated following (Phillips 1959). Specimens were collected during growing season from study site and identified from Division of Environmental science, SKUAST-Kashmir and centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir. The data on vegetation was quantitatively analysed for density, basal area, frequency, Importance value index (IVI) separately for three different life forms i.e. tree, shrub and herbaceous species (Misra 1969, Phillips 1959).

$$\text{Density} = \frac{\text{Total number of individuals of a species in all quadrates}}{\text{Total number of quadrates studied}}$$

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrates studied}} \times 100$$

$$\text{Basal area} = \frac{na^2}{4}, \text{ where } d = \text{Diameter of tillers}$$

**Importance value index (IVI)** = Relative density + Relative frequency + Relative basal area

## RESULTS AND DISCUSSION

The highest average height (23.91 m) was recorded for trees in *Robinia pseudoacacia* based agroforestry system followed by the *Cupressus torulosa* (22.33 m). Trees of *Prunus arminiaca* system had the shortest average height (6.95 m) and the grassland did not have any trees (Table 1). The highest average diameter at breast height (dbh) of trees occurred in the *Cupressus torulosa* based system (91.72 cm), while the lowest dbh was in mixed stand (19.91 cm/tree).

The density of shrub species (small, medium and large) in these agroforestry systems ranged from 133.33 to 1200 shrubs/ha (Fig. 1-3). Broad-leaved trees supported a higher number of shrub species, potentially leading to greater shrub

occurrences in *Prunus arminiaca* system. Similarly, herbaceous density was highest in grassland (1239999 tillers ha<sup>-1</sup>), with the lowest herb density in *Cedrus deodara* (169999 tillers ha<sup>-1</sup>) (Tables 2-8).

The current investigation, aimed at assessing variations in shrub and herbage growth under different agroforestry systems indicated a decreasing density of shrubs in the order grassland (control) > *Prunus arminiaca* > *Robinia pseudoacacia* > *Cupressus torulosa* > *Ailanthus altissima* > mixed stand > *Cedrus deodara*. Likewise, the density of herbs decreased in the order: grassland (control) > *Prunus arminiaca* > *Robinia pseudoacacia* > *Ailanthus altissima* > *Cupressus torulosa* > mixed stand > *Cedrus deodara*. This trend was attributed to the reduction in relative light intensity under trees by various studies (Guleria et al., 1999, Naugraiya and Pathak 2001 and Ludwig et al., 2004). The low density of vegetation under tree canopies due to changes in microclimate, light interception, allelochemicals release and other inhibitory effects. Strong competition for space and light accompanied by release of allelochemicals by trees like *Ailanthus altissima* might have prohibited the propagation of shrubs and herbages in these systems. Difference in grassland (control) and herbages in plantations were regulated by few ubiquitous species. Least number of species in deodar plantation was due to pine needle litter deposition on the forest floor which might have restricted germination of herbaceous flora (Gupta et al., 2007, Dangwal et al., (2012). Barbier et al., (2008) concluded that mixing of deciduous and coniferous tree species generally affects understory diversity, but in almost all cases maximum diversity is observed in one of the pure stands, not in mixed stands. Floristic composition is a measure of species diversity in a community and t is a long term process to give clear cut information of species diversity of an area as it is liable to change with season as well as the effect of biotic factors, microclimate and their interaction in any community (Husain et al., 2019). Microclimatic conditions could varies more widely in deciduous and mixed forest stands than in evergreen stands, in response to the annual gradient of light, influencing soil properties (Gazol and Ibanez 2010, Yu and Sun 2013, Marialigeti et al., 2016).

In the *Cedrus deodara* plantation, *Cyanodon dactylon* and *Salvia moorcroftiana* exhibited maximum dominance over other herbage species, categorizing the community as Cyanodon-Salvia type. In *Robinia pseudoacacia* and *Prunus arminiaca* plantations, *Lolium perenne* and *Cynodon dactylon* displayed maximum dominance, designating the community as *Lolium-Cyanodon* type. Similarly, *Salvia moorcroftiana* and *Cyanodon dactylon* recorded the highest Importance value index (IVI) in *Cupressus torulosa* and

**Table 1.** Growth parameters of tree species and light intensity in different agroforestry systems of Ganderbal Kashmir

Growth parameters		T1	T2	T3	T4	T5	T6	T7
Height (m)	Minimum	16	18	15.2	4.2	5	4	-
	Maximum	24.5	28.6	29.7	9.4	28	18	-
	Standard error	0.29	0.48	0.97	0.18	0.78	0.48	-
	Standard deviation	2.28	2.96	4.32	1.26	6.18	4.34	-
Dbh (cm)	Minimum	27	22	52	8	6	6	-
	Maximum	92	46	132.2	44	91	34	-
	Standard error	2.11	1.07	5.62	1.38	3.02	0.88	-
	Standard deviation	16.20	6.65	25.14	9.96	23.98	7.86	-
Relative light intensity (%)		21.81	58.21	45.24	69.16	47.85	24.76	100.00

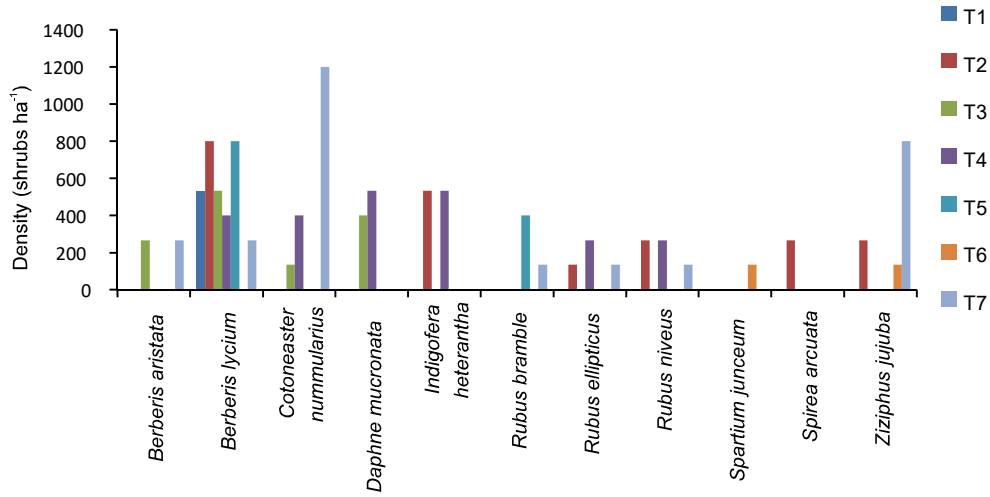
T1-*Cedrus deodara*;T2- *Robinia pseudoacacia*;T3- *Cupressus torulosa*;T4- *Prunus armeniaca* ;T5-*Ailanthus altissima*; T6- mixed stand (*Cupressus torulosa*, *Robinia pseudoacacia*, *Ailanthus altissima*;T7-grassland(control)

**Table 2.** Phytosociological attributes of herbs in *Cedrus deodara* based agroforestry system

Species name	Relative density (%)	Relative frequency (%)	Relative basal area (%)	Importance value index (%)
<i>Asplenium species</i>	8.47	12.50	0.53	22.84
<i>Cyanodon dactylon</i>	15.25	12.50	39.31	69.45
<i>Oxalis acetosella</i>	16.95	12.50	7.74	39.85
<i>Oxalis corniculata</i>	6.78	12.50	3.21	23.55
<i>Plantago lanceolata</i>	11.86	12.50	15.51	41.73
<i>Salvia moorcroftiana</i>	13.56	12.50	25.17	53.35
<i>Stipa sibirica</i>	6.78	12.50	4.27	24.62
<i>Trifolium pretense</i>	6.78	12.50	4.26	24.61

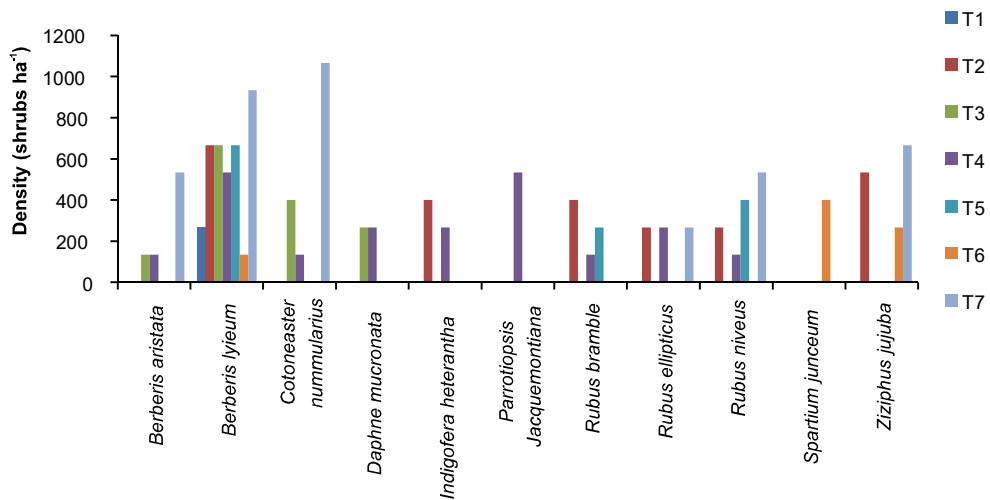
**Table 3.** Phytosociological attributes of herbs in *Robinia pseudoacacia* based agroforestry system

Species name	Relative density (%)	Relative frequency (%)	Relative basal area (%)	Importance value index (%)
<i>Amaranthus viridis</i>	4.68	6.38	12.29	23.36
<i>Asplenium species</i>	7.81	6.38	6.38	20.57
<i>Bothriochloa ischaemum</i>	7.03	6.38	1.92	15.34
<i>Centaurea iberica</i>	7.03	6.38	10.61	24.03
<i>Chenopodium axanthum</i>	4.68	6.38	5.02	16.09
<i>Conyza Canadensis</i>	3.12	4.26	3.68	11.06
<i>Cynodon dactylon</i>	10.93	6.38	8.96	26.28
<i>Lolium perenne</i>	21.09	6.38	18.58	46.05
<i>Medicago minima</i>	4.68	6.38	3.85	14.92
<i>Plantago lanceolata</i>	3.90	6.38	6.65	16.94
<i>Plantago major</i>	7.81	6.38	6.57	20.76
<i>Poa annua</i>	3.12	6.38	2.55	12.06
<i>Poa bulbosa</i>	3.90	6.38	3.35	13.63
<i>Scandix pectenvenersis</i>	3.90	6.38	4.49	14.78
<i>Stipa sibirica</i>	3.12	6.38	2.57	12.08
<i>Trifolium pretense</i>	3.12	6.38	2.56	12.06



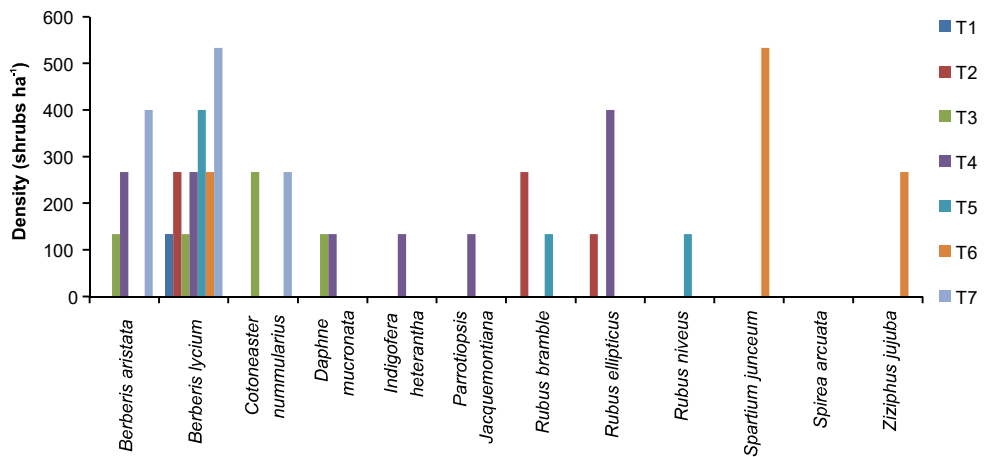
T1-*Cedrus deodara*;T2- *Robinia pseudoacacia*;T3- *Cupressus torulosa*;T4- *Prunus arminiaca* ;T5-*Ailanthus altissima*; T6- mixed stand (*Cupressus torulosa*, *Robinia pseudoacacia*, *Ailanthus altissima*);T7-grassland(control)

Fig. 1. Density of small sized shrubs in different agroforestry systems of Kashmir



(See Fig 1 for details)

Fig. 2. Density of medium sized shrubs in different agroforestry systems of Kashmir



(See Fig 1 for details)

Fig. 3. Density of large sized shrubs in different agroforestry systems of Kashmir

**Table 4.** Phytosociological attributes of herbs in *Cupressus torulosa* based agroforestry system

Species name	Relative density (%)	Relative frequency(%)	Relative basal area (%)	Importance value index (%)
<i>Centaurea iberica</i>	5.48	11.54	8.10	26.20
<i>Chenopodium album</i>	5.48	11.54	4.40	22.50
<i>Cynodon dactylon</i>	16.44	11.54	13.19	44.40
<i>Frageria vesca</i>	5.48	11.54	4.42	22.51
<i>Marubium vulgare</i>	5.48	7.69	7.22	21.47
<i>Oxalis acetosella</i>	9.59	11.54	7.85	30.87
<i>Oxalis corniculata</i>	9.59	11.54	8.17	31.18
<i>Salvia moorcroftiana</i>	16.44	11.54	38.93	70.14
<i>Stipa sibirica</i>	9.59	11.54	7.72	30.73

**Table 5.** Phytosociological attributes of herbs in *Prunus armeniaca* based agroforestry system

Species name	Relative density (%)	Relative frequency(%)	Relative basal area (%)	Importance value index (%)
<i>Achillea millefolium</i>	1.73	2.86	1.51	6.09
<i>Agrimonia eupatoria</i>	2.88	2.86	4.30	10.03
<i>Amaranthus caudatus</i>	1.73	2.86	3.85	8.43
<i>Amaranthus viridis</i>	2.02	2.86	4.65	9.53
<i>Arctium lappa</i>	2.88	2.86	6.72	12.46
<i>Artemisia absinthium</i>	1.44	2.86	1.03	5.33
<i>Arnebia hispidissima</i>	1.44	2.86	2.71	7.01
<i>Asplenium species</i>	1.73	2.86	1.24	5.83
<i>Bothriochloa ischaemum</i>	2.88	2.86	0.69	6.43
<i>Cichorium intybus</i>	2.88	2.86	2.09	7.83
<i>Conyza Canadensis</i>	2.88	2.86	2.98	8.72
<i>Cynodon dactylon</i>	6.05	2.86	4.36	13.27
<i>Daucus carota</i>	2.31	2.86	2.89	8.05
<i>Fragaria nubicula</i>	2.02	2.86	1.46	6.33
<i>Frageria vesca</i>	1.15	2.86	1.10	5.11
<i>Lespedeza species</i>	4.03	2.86	3.02	9.91
<i>Lolium perenne</i>	7.49	2.86	5.81	16.16
<i>Malva neglecta</i>	2.31	2.86	1.66	6.82
<i>Marubium vulgare</i>	1.44	2.86	1.70	6.00
<i>Medicago minima</i>	3.17	2.86	2.29	8.32
<i>Plantago lanceolata</i>	2.59	2.94	4.46	10.15
<i>Plantago major</i>	4.03	2.94	3.43	10.65
<i>Rumex nepalensis</i>	2.59	2.94	2.15	7.84
<i>Salvia moorcroftiana</i>	4.03	2.94	2.29	9.52
<i>Scandix pectenvenensis</i>	3.46	2.94	4.02	10.63
<i>Setaria viridis</i>	3.75	2.94	2.28	9.20
<i>Solanum nigrum</i>	2.31	2.94	2.07	7.46
<i>Sorghum helpense</i>	3.75	2.94	2.31	9.23
<i>Stipa sibirica</i>	1.15	2.94	0.96	5.12
<i>Taraxicum officinale</i>	2.02	2.94	1.67	6.75
<i>Trifolium pretense</i>	3.46	2.94	2.86	9.47
<i>Trifolium repens</i>	2.02	2.94	1.67	6.75
<i>Urtica dioica</i>	2.59	2.94	3.86	9.55
<i>Viola odorata</i>	2.02	2.94	1.66	6.74



**Table 6.** Phytosociological attributes of herbs in *Ailanthus altissima* based agroforestry system

Species name	Relative density (%)	Relative frequency(%)	Relative basal area (%)	Importance value index (%)
<i>Arctium lappa</i>	4.55	8.57	12.83	27.20
<i>Centaurea iberica</i>	3.41	5.71	5.48	15.55
<i>Chenopodium album</i>	4.55	8.57	3.97	18.34
<i>Conyza Canadensis</i>	5.68	8.57	7.12	22.94
<i>Cynodon dactylon</i>	11.36	8.57	9.92	32.98
<i>Frageria vesca</i>	4.55	8.57	3.98	18.35
<i>Lespedeza species</i>	9.09	8.57	8.24	28.41
<i>Medicago minima</i>	4.55	8.57	3.97	18.34
<i>Poa annua</i>	3.41	5.71	2.96	13.03
<i>Poa bulbosa</i>	5.68	5.71	5.19	18.15
<i>Salvia moorcroftiana</i>	9.09	8.57	23.41	43.58
<i>Scandix pectenvenersis</i>	5.68	5.71	6.97	19.93
<i>Trifolium pratense</i>	6.82	8.57	5.95	23.21

**Table 7.** Phytosociological attributes of herbs in mixed tree based agroforestry system

Species name	Relative density (%)	Relative frequency(%)	Relative basal area (%)	Importance value index (%)
<i>Agrimonia eupatoria</i>	7.55	13.64	15.08	36.26
<i>Bothriochloa ischaemum</i>	5.66	9.09	1.83	16.58
<i>Cichorium intybus</i>	5.66	9.09	5.50	20.25
<i>Cynodon dactylon</i>	15.09	13.64	14.58	43.31
<i>Lespedeza species</i>	9.43	13.64	9.46	32.53
<i>Lolium perenne</i>	24.53	13.64	23.76	61.92
<i>Oxalis corniculata</i>	20.75	13.64	21.56	55.95
<i>Stipa sibirica</i>	11.32	13.64	8.23	33.19

*Ailanthus altissima* system, classifying the community as Salvia-Cyanodon type. In mixed stand, *Lolium perenne* and *Oxalis corniculata* dominated, making it a *Lolium-Oxalis* type community. Lastly, in grassland (control), *Salvia moorcroftiana* and *Lolium perenne* exhibited maximum dominance, categorizing the community as *Salvia-Lolium* type. *Berberis lycium* was common in all agroforestry systems, while among herbaceous species *Cynodon dactylon* was observed in all systems. The dominance of specific species in each community was attributed to their adaptability and growth in particular environments (Gupta et al., 2002). Understorey species composition in these forests differed which is a manifestation of type (species and density) of over storey trees and their influence likely to occur on herbaceous layer diversity by modifying resource availability and environmental conditions relevant to herbages (Vockenhuber et al., 2011, Manzoor and Jazib (2020). The importance of canopy-species leaf litter as a key factor influencing soil acidity and thereby nutrient stocks, whereas the upper 10 cm of soil are most significantly influenced by

tree-species effects (Augusto et al., 2003). Additionally, thickness of litter layer varies according to tree species (Augusto et al., 2002).

### CONCLUSION

The herbaceous growth showed strong dependence on light intensity, density of trees and canopy cover. There was significant decrease in functional parameters of herbs under trees as compared to grassland. *Cynodon dactylon* was the dominating species under grassland (control), *Ailanthus altissima* and *Cupressus torulosa* plantations. *Oxalis acetosella* was dominating under *Cedrus deodara* plantations. *Lolium perenne* was dominating species under mixed stand, *Robinia pseudoacacia* and *Prunus armeniaca* plantations. The type of overstorey trees had insignificant influence on shrub and herbaceous composition but significantly influenced the herbaceous growth. The magnitude of density, basal area and biomass of herbs and shrubs under different trees was less in comparison to grassland (control).

**Table 8.** Phytosociological attributes of herbs in grass land based system

Species name	Relative density (%)	Relative frequency(%)	Relative basal area (%)	Importance value index (%)
<i>Achillea millefolium</i>	1.28	2.88	1.18	5.41
<i>Agrimonia eupatoria</i>	2.82	2.88	4.44	10.28
<i>Amaranthus caudatus</i>	1.79	1.92	4.22	8.02
<i>Amaranthus viridis</i>	2.31	2.88	5.62	10.93
<i>Artemisia absinthium</i>	1.79	2.88	1.36	6.12
<i>Arnebia hispidissima</i>	1.54	2.88	3.05	7.55
<i>Bothriochloa ischaemum</i>	3.08	2.88	0.78	6.89
<i>Centaurea iberica</i>	2.05	2.88	2.88	7.91
<i>Chenopodium album</i>	2.05	2.88	1.56	6.60
<i>Chenopodium axanthum</i>	1.54	2.88	1.53	6.03
<i>Cichorium intybus</i>	3.33	2.88	3.07	9.45
<i>Conyza Canadensis</i>	2.31	2.88	3.63	8.94
<i>Cynodon dactylon</i>	6.67	1.92	5.07	13.99
<i>Cymbopogon nardus</i>	1.03	2.88	1.55	5.51
<i>Daucus carota</i>	1.54	2.88	1.55	6.05
<i>Frageria vesca</i>	2.05	2.88	4.07	9.11
<i>Lespedeza species</i>	5.13	2.88	4.05	12.31
<i>Lolium perenne</i>	6.15	2.88	5.04	14.37
<i>Malva neglecta</i>	2.56	2.88	1.95	7.52
<i>Medicago minima</i>	3.08	2.88	2.35	8.46
<i>Plantago lanceolata</i>	2.56	2.88	4.06	9.63
<i>Plantago major</i>	1.54	2.88	1.20	5.70
<i>Poa annua</i>	1.28	1.92	0.97	4.24
<i>Poa bulbosa</i>	1.03	2.88	0.82	4.78
<i>Poa pretense</i>	1.03	2.88	0.71	4.67
<i>Rumex nepalensis</i>	4.10	2.88	3.13	10.32
<i>Salvia moorcroftiana</i>	4.36	2.88	9.79	17.24
<i>Scandix pectenvenersis</i>	3.33	2.88	3.56	9.94
<i>Setaria viridis</i>	4.62	2.88	2.62	10.34
<i>Sorghum helpense</i>	4.10	2.88	2.33	9.52
<i>Stipa sibirica</i>	0.77	2.88	0.59	4.28
<i>Taraxicum officinale</i>	2.05	1.92	1.56	5.64
<i>Trifolium pratense</i>	3.33	2.88	2.53	8.91
<i>Trifolium repens</i>	2.82	2.88	2.14	7.99
<i>Urtica dioica</i>	2.82	2.88	3.86	9.70
<i>Viola odorata</i>	1.54	2.88	1.17	5.66

#### AUTHOR'S CONTRIBUTION

TAR and AS initiated and conceptualized the study. All authors (TAR, AS, AHM, BGM, AAM, MRB) contributed to field data collection and lab work. TAR, AS and AHM contributed to data evaluation. Authors (TAR, AS) contributed to writing and reviewing the manuscript.

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# Assessing Population Dynamics and Seed Germination in the Endemic Tree *Micromeles cuspidata* (Bertol.) C.K. Schneid.

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**Abstract:** The present study assessed the population status and seed germination of the endemic tree *Micromeles cuspidata* (Bertol.) C.K. Schneid. in northeast India. A total population density of 594 individuals was recorded, consisting of 67 adults, 215 saplings, and 312 seedlings. Regeneration was generally good across sites, except for sites Law Shnong Mawkasain and Law Shnong Umladkur, where no saplings or seedlings were present. Sites Law Adong Pongtung and Syiemship forest demonstrated fair regeneration. Seed germination experiments revealed that all treatments induced germination within two weeks. The highest germination rate (96%) occurred in seeds treated with 2000 mg L<sup>-1</sup> gibberellic acid (GA<sub>3</sub>), while the lowest rate (65.33%) was observed at 3000 mg L<sup>-1</sup> GA<sub>3</sub>, indicating that higher concentrations had inhibitory effects. Untreated seeds also exhibited a high germination rate (90.67%), suggesting that *M. cuspidata* can be propagated effectively without chemical intervention. These results provide critical insights for ex-situ conservation strategies. The conservation of adult trees in natural habitats is essential for maintaining viable populations in the wild. Furthermore, seed collection, germination and the reintroduction of seedlings into community-conserved areas are recommended to enhance the species' long-term survival.

**Keywords:** Conservation, Ex-situ, Gibberellins, Germination, Population density

*Micromeles cuspidata* (Bertol.) C.K. Schneid. (Synonyms- *Photinia cuspidata*, *Pyrus cuspidata*, *Sorbus verrucosa* and *Micromeles verrucosa*), is a small tree species of the family Rosaceae. The species is distributed in tropical and subtropical forests of India and parts of Southeast Asia. In India, the species has been recorded from a few hill forests in Meghalaya (Tripathi 2013, Mir 2017) and is considered endemic to the region (Upadhaya et al., 2017). However, the species' population is small and faces threats from anthropogenic activities and habitat degradation. The current trends suggest a continued decline in its population unless conservation measures are urgently implemented (Mir and Upadhaya 2021). The species is harvested locally as a good source of firewood (Mir 2017). The International Union for Conservation of Nature (IUCN) has categorized the species as 'Data Deficient' highlighting the paucity of data on the status of the species beyond Meghalaya (IUCN 2021). Furthermore, no conservation initiatives have been undertaken for this species, and it remains absent from any ex-situ collections (BGCI 2019). Thus, this study seeks to assess the population status of the tree species in Meghalaya and study seed germination potential of *M. cuspidata* to facilitate ex-situ propagation and conservation efforts of this vulnerable species.

## MATERIAL AND METHODS

**Study area:** The study was carried out in the Khasi and Jaintia Hills of Meghalaya (89°49'E to 92°50'E longitude and

25°02'N to 26°07'N latitude). The elevation ranges from 900-1500 m asl. The forest types found in this region are categorized as subtropical broad-leaved humid forests (Champion and Seth 1968). The average annual rainfall is ca. 3500 mm and temperatures vary from 26°C in summer to 5°C in winter.

**Population structure:** Extensive field surveys were conducted in the potential areas of the species occurrence based on herbarium records. The species was found in 18 sites spread across the Khasi and Jaintia Hills of Meghalaya. To understand the population density of the species in these forests, a belt transect measuring 20 m wide and 250 m long was laid in each forest. The transect was further divided into 10 m<sup>2</sup> quadrates for sampling of *M. cuspidata* and associated species where all trees measuring ≥ 5 cm diameter at breast height (dbh) were counted and measured. Individuals <5 cm dbh and > 1 m height were categorized under saplings and those <1 m height were categorized as seedlings. The regeneration was categorized as good, fair, poor, none and new following the Sukumar et al. (1992) based on the number of trees, saplings and seedlings.

**Seed source:** Mature fruits of *M. cuspidata* were collected from Mawsynram region (25.298°N, 91.581°E) in Meghalaya in mid-January. Fruits were collected from randomly selected trees to attain a composite and representative seed lot. The fruits measured approximately 0.87 cm in diameter and typically contained 3 to 4 ovate seeds, each measuring 3.9 mm across. The seeds were manually extracted and

thoroughly washed and air-dried. The seeds were stored at room temperature ( $24 \pm 1^\circ\text{C}$ ). Germination experiments were conducted within one week of seed collection to avoid the loss of viability.

**Germination tests:** Seed germination rates were systematically evaluated under controlled laboratory conditions to determine the impact of  $\text{GA}_3$  treatments. Seeds were subjected to a 48-hour soaking period in a range of  $\text{GA}_3$  solutions with concentrations of  $200 \text{ mg L}^{-1}$ ,  $500 \text{ mg L}^{-1}$ ,  $1000 \text{ mg L}^{-1}$ ,  $2000 \text{ mg L}^{-1}$ , and  $3000 \text{ mg L}^{-1}$ . The control group was maintained where seeds received no  $\text{GA}_3$  treatment for comparison with  $\text{GA}_3$  treated seeds. Both the control and  $\text{GA}_3$ -treated seeds were placed on glass Petri dishes that measured  $9 \text{ cm diameter} \times 2 \text{ cm height}$  lined with Whatman No. 1 filter paper. The seeds were incubated at a constant temperature of  $25^\circ\text{C} (\pm 1^\circ\text{C})$ . For each  $\text{GA}_3$  concentration, three replicates, each with 25 seeds, were maintained. The filter papers were regularly moistened with distilled water at 3-day intervals to maintain moisture levels. Seed germination was carefully monitored daily until the cessation of germination. To understand seedling growth, 500 seeds were sown in plastic trays filled with a mixture of soil and sand at a ratio of 3:1. The germinated seedlings were transplanted into poly bags after 3 months and transferred to greenhouse conditions having a controlled light intensity of approximately  $3 \text{ mol m}^{-2} \text{ d}^{-1}$ , similar to understory light intensities in the field conditions. The seedlings were watered at 3-day intervals and mortality rates were documented. After one year, 10 seedlings were harvested to assess the growth in shoots, roots, leaves and biomass.

**Data analysis:** The germination percentage for each treatment replication was determined.

$$G(\%) = n/N \times 100,$$

Where  $n$  represents the number of seeds that germinated, and  $N$  denotes the total number of seeds.

Mean germination time (MGT) was calculated using the following formula:

$$\text{MGT} = \sum (n * d) / N,$$

where  $n$  is the number of seeds that germinated on a particular day,  $d$  is the number of days since the test began, and  $N$  is the total number of seeds that germinated by the end of the experiment (Ellis and Roberts 1981). The time required for 50% germination ( $T_{50}$ ) was calculated as follows:

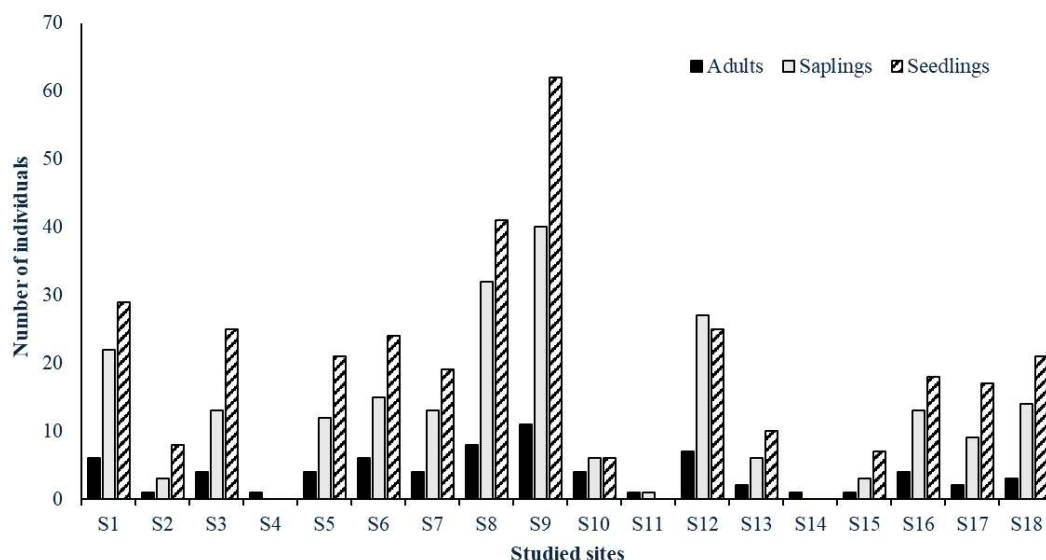
$$T_{50} = t_1 + [(N/2 - n_1)(t_2 - t_1)] / (n_2 - n_1),$$

where  $N$  is the final number of germinated seeds,  $n_1$  and  $n_2$  are the cumulative numbers of germinated seeds at times  $t_1$  and  $t_2$ , respectively, when  $n_1 < N/2 < n_2$  (Farooq et al., 2006).

The analysis was performed with Tukey's least significant difference test ( $p < 0.05$ ) using SPSS (Version 20).

## RESULTS AND DISCUSSION

**Population structure:** The total population density was 594 individuals comprising 67 adults, 215 saplings and 312 seedlings. The highest number of individuals were observed in Sai Mika forest (S9) with 113 individuals and the lowest population was in Law Shnong Mawkasain (S4) and Law Shnong Umladkur (S14) with only 1 adult individual in each site (Table 1, Fig. 1). The dominant associated species were *Castanopsis tribuloides* (Sm.) A. DC., *Castanopsis purpurella* (Miq.) N.P. Balakr., *Cinnamomum pauciflorum* Nees, *Elaeocarpus lancifolius* Roxb. and *Helicia nilagirica* Bedd. The regeneration of the species based on the number of adults, saplings and seedlings revealed an overall good regeneration except for sites S4 and S14 where saplings and



**Fig. 1.** Number of adults, saplings and seedlings of *M. cuspidata* in the studied sites

seedlings were absent. Two sites S10 and S12 showed fair regeneration (Table 1).

The variation in the population density of *M. cuspidata* in different sites may be attributed to differences in land use, topography and anthropogenic activities operating in and around the habitat of the species. The species was in both forest edges and in dense forests indicating the species' ability to withstand external disturbances. However, as the species is harvested for its valued firewood, forest patches located close to villages are at higher risk of over-exploitation. Studies have shown that disturbance poses the risk of reducing the densities of flowering species risking the proliferation of invasive species (Bisht et al., 2022). Disturbance in the form of extraction is known to reduce the density of flowering trees as observed in species such as *Magnolia rabaniana* (Mir et al., 2017). The regeneration of the species was satisfactory across most of the studied sites, as indicated by a higher number of saplings and seedlings compared to adults. The highest number of adult trees was at site S9, with 11 individuals, although the overall number of adults across all sites remained low. The positive correlation was observed between the densities of saplings, seedlings and adult trees, underscoring the importance of conserving adult populations in their natural habitats to ensure the long-term persistence of this endemic species.

**Seed germination:** Seed germination was observed within 14.5 days after the start of the experiment. The average

germination percentage was 87.11 across the treatments. MGT was 13.50-days and the time to reach 50% germination ( $T_{50}$ ) was 14.97. Seeds that were not subjected to GA<sub>3</sub> treatment showed a relatively high germination rate of 90.67%, with an MGT of 14.96 days and a  $T_{50}$  of 15.67 days. The highest germination percentage of 96 was in seeds treated with 2000 mg L<sup>-1</sup> GA<sub>3</sub>, which also had the shortest MGT of 11.85 days (Table 1). However, seeds treated with 3000 mg L<sup>-1</sup> GA<sub>3</sub> resulted in the lowest germination percentage (65.33) which was significantly lower than all other treatments. This treatment also had the longest MGT (15.21 days) albeit the  $T_{50}$  was the shortest (13.54 days). Seeds treated with 1000 mg L<sup>-1</sup> GA<sub>3</sub> had the longest  $T_{50}$  (16.41 days). The application of GA<sub>3</sub> expedited the time for germination in seeds with significantly shorter mean germination days achieved in seeds treated with higher concentrations of GA<sub>3</sub> as compared to untreated seeds (Table 2, Fig. 2). Seedlings transplanted into greenhouse conditions had a survival of 55% after one year. Seedlings were vulnerable to desiccation and seedling growth was slow (Table 3).

Seeds of *M. cuspidata* exhibited high percentages of germination for both treated and untreated seeds under controlled laboratory conditions. Seeds germinated within two weeks indicating the absence of dormancy at the time of seed dispersal. The GA<sub>3</sub> treatments showed variations in the germination percentage with the lowest percentage

**Table 1.** Basal area of adult *M. cuspidata* in the studied sites and regeneration status

Sites	Site code	Density of adult trees	Basal area (m <sup>2</sup> )	Regeneration status
Law Adong Laitsohum	S1	6	0.565	Good
Law Adong Saitbakon	S2	1	0.002	Good
Law Kyntang Nonglienkien	S3	4	0.040	Good
Law Shnong Mawkasain	S4	1	0.001	None
Twah Samparat	S5	4	0.042	Good
Tyllong Um-Kyrwiang	S6	6	0.050	Good
Law Adong Tyrsad	S7	4	1.010	Good
Law Adong Phlangwanbroi	S8	8	0.260	Good
Sai Mika	S9	11	0.116	Good
Syiemship forest RK Mission	S10	4	0.233	Fair
Lum Shynna	S11	1	0.045	Poor
Law Adong Pongtung	S12	7	0.325	Fair
Law Siarpa	S13	2	0.342	Good
Law Shnong Umladkur	S14	1	0.093	None
Tyrongmawlieh Mission Tynnai	S15	1	0.039	Good
Law Kyntang Tyrsad	S16	4	0.505	Good
Law Marai	S17	2	0.202	Good
Wah Bah Pomolang	S18	3	0.150	Good

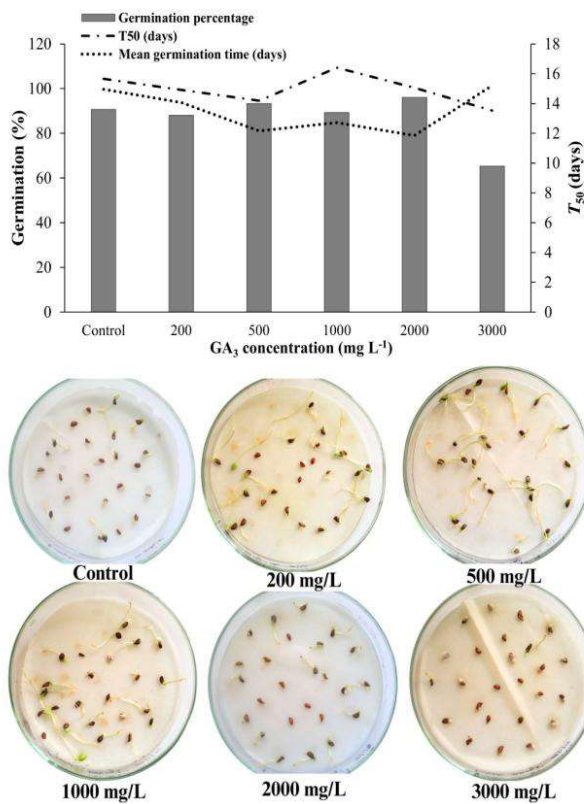
**Table 2.** Germination characteristics of *M. cuspidata* seeds

Treatment (mg L <sup>-1</sup> )	Total germinated	Germination percentage	Mean germination time (days)	T <sub>50</sub> (days)
Control	22.67±0.33 <sup>a</sup>	90.67±1.33 <sup>a</sup>	14.96±0.04 <sup>a</sup>	15.67±0.09 <sup>a</sup>
200	22.00±0.00 <sup>a</sup>	88.00±0.00 <sup>a</sup>	14.07±0.20 <sup>ab</sup>	14.92±0.08 <sup>ab</sup>
500	23.33±0.33 <sup>a</sup>	93.33±1.33 <sup>a</sup>	12.17±0.05 <sup>d</sup>	14.19±0.06 <sup>b</sup>
1000	22.33±0.33 <sup>a</sup>	89.33±1.33 <sup>a</sup>	12.73±0.26 <sup>d</sup>	16.41±0.57 <sup>a</sup>
2000	24.00±0.58 <sup>a</sup>	96.00±2.31 <sup>a</sup>	11.85±0.39 <sup>d</sup>	15.06±0.42 <sup>a</sup>
3000	21.78±0.62	65.33±1.33	15.21±0.22 <sup>ac</sup>	13.54±0.12 <sup>b</sup>

Means followed by the same letter in each column do not differ significantly at  $p < 0.05$ .

**Table 3.** Growth characteristics of one-year-old *M. cuspidata* seedlings (n=10)

Light intensity	No. of leaves	Mean leaf area (cm <sup>2</sup> )	Shoot height (cm)	Root length (cm)	Dry weight (g)
3 ± 0.35 mol m <sup>-2</sup> d <sup>-1</sup>	8±1	6.90±0.36	11.45±0.76	12.53±1.03	0.22±0.04

**Fig. 2.** Mean germination percentage (G%), MGT and T<sub>50</sub> under GA<sub>3</sub> treatments at 25°C and seeds germinating under the various GA<sub>3</sub> concentrations

observed in the highest concentration of 3000 mg L<sup>-1</sup>. This observation suggests the inhibitory effect of GA<sub>3</sub> at high concentrations. Similar observation was observed in other species like *Saraca asoca* and *Melientha suavis* (Rout et al., 2021, Tuan et al., 2023). High GA<sub>3</sub> concentrations above species threshold limits can lead to disruptions in hormonal signaling and seed metabolism (Attia et al., 2022). The application of GA<sub>3</sub> expedited the germination process, with 2000 mg L<sup>-1</sup> observed as the most effective concentration for

achieving rapid and high germination. Similar results have also been reported in species such as *Magnolia punduana* and *Tinospora cordifolia* (Iralu and Upadhaya 2016, Bhadra et al., 2024). Untreated seeds also achieved high germination percentages suggesting that the species can be propagated without external treatments. For conservation purposes, it is recommended that adult trees be given conservation priority. In addition, ex-situ conservation through mass germination and the introduction of seedlings in botanical gardens, national parks and community-conserved areas will go a long way in ensuring the conservation of the species.

## CONCLUSION

*Micromeles cuspidata* showed good regeneration in most sites, with significant numbers of saplings and seedlings, highlighting the potential for recovery if habitat degradation is controlled. The low density of adult individuals, especially in areas heavily impacted by anthropogenic activities, emphasizes the need for urgent conservation actions. High germination rates achieved in both untreated and GA<sub>3</sub>-treated seeds suggest that the species can be effectively propagated through seed-based approaches. Despite challenges in seedling establishment under greenhouse conditions, the successful germination and propagation of *M. cuspidata* offers a viable path for ex-situ conservation efforts. Priority should be given to protecting adult populations and implementing conservation strategies, including introducing seedlings into botanical gardens and forest areas, to ensure the long-term survival of this endemic species.

## ACKNOWLEDGEMENTS

The financial support received from the University Grants Commission (UGC) in the form of RGNF fellowship (Grant No.: F1-17.1/2013-14/RGNF-2013-14-ST-NAG-43868/(SA-III/website) is acknowledged. I am also thankful to the village

heads of the Khasi Hills for permitting me to work and collect samples in their village forests.

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Received 11 October, 2024; Accepted 24 January, 2025





# Distribution of *Sphyraena forsteri* in the Arabian Sea off South Gujarat

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**Abstract:** The objective of the present study was to provide classical taxonomic analysis and a detailed description of *Sphyraena forsteri* a specimen obtained through trawling operation and brought to Dholai fishing harbour. The single specimen of 515 mm length and 210 gm weight was collected for morphometric analysis for the first time from Dholai fishing harbour along the Arabian Sea coast of the South Gujarat region. This paper provides comprehensive details about the morphometric parameters observed in adult specimen of *S. forsteri*.

**Keywords:** Barracudas, Dholai Fishing Harbour, South Gujarat

The 27 extant species of barracudas (family Sphyraenidae) are one of the major groups of large coastal piscivores in tropical and subtropical marine habitats. Most barracudas are found in coastal areas, often in close association with stony coral reefs, but several species have a more pelagic distribution and are known to cross large tracts of open water (Daly Engel et al., 2012). Barracudas tend to be opportunistic predators, with a hunting technique that relies on ambushing prey thanks to their ability to sustain bursts of high swimming speed over short distances. Most species are known to form schools as either juveniles or adults, with schools containing hundreds of individuals in some cases. Some species, however, tend to be solitary or only live in small groups, and adult individuals of some schooling species can sometimes live solitary lives (Froese and Pauly 2014).

Traditionally, barracudas were thought to be related to tunas, snake mackerels, and allies, and, based on selected morphological characters, they have been suggested as the sister group to all remaining members of the perciform suborder Scombroidei. Numerous molecular analyses have definitively refuted the notion of Scombroidei as a monophyletic group (Betancur-R et al., 2013, Miya et al., 2013, Near et al., 2013). These studies reveal that Sphyraenidae, traditionally classified within Scombroidei, are instead part of a larger assemblage that encompasses jacks and pompanos (Carangiformes), flatfishes (Pleuronectiformes), and several other lineages previously considered unrelated such as archerfishes (Toxotidae) (Santini et al., 2014).

Throughout India in the year 2020-21, a total of 37,749 tons of barracudas were landed (Gopalkrishna 2021). The

major species landed are *Sphyraena obtusata*, *S. jello*, *S. barracuda*, and *S. acutipinnis* with the first two species together constituting over 50% of barracuda landings. Multiday trawlers account for nearly 75% of the barracudas landed followed by outboard gillnetters (Abdul et al., 2021). Globally, most cases of incidental capture of *S. forsteri* in shrimp trawl fisheries indicate that it is not specifically targeted or heavily exploited in these fisheries. This incidental capture does not appear to pose a significant threat to the overall population of *S. forsteri*, as it is not being subjected to intense fishing pressure. Furthermore, *S. forsteri* is not the primary target of the trawl fishery suggests that its population is not being disproportionately impacted by fishing activities, thus it is categorized as 'Least concern (LC)' in the IUCN Red List (IUCN 2024).

The state of Gujarat, with its 1600 km coastline, accounts for approximately 26% of the total mainland coastline of India, yet it plays a significant role in fish production. Due to the natural topography on the west coast, the majority of these harbours and landing centers are situated along the northwest coast of Gujarat. Only one fishing harbour, Dholai, and six fish landing centers are located on the southwest coast of Gujarat. Dholai fishing harbour, situated in the Navsari district, serves as a major fisheries hub in the south Gujarat region. Most of the vessels registered at the harbour are mechanized trawlers, with a few exceptions such as small gill netters and other dug-out canoes. The objective of the current study is to provide a detailed description of *S. forsteri*, including its taxonomic classification and morphological characteristics.

## MATERIAL AND METHODS

The sample was collected from Dholai fishing harbour (20.73°N, 72.89°E) and brought to the College of Fisheries Science, Navsari and was washed of any debris and other materials. The specimen was observed and measured for various morphometric parameters and meristic characters (Table 1). Identification was done using FAO species identification sheets (Fischer 1984) and Commercial Sea Fishes of India (Kacker and Talwar 1984) The specimen was photographed and submitted to the Aquatic Biodiversity Museum where it was preserved in 10% formalin (Accession No: A 15.15.1.2)

## RESULTS AND DISCUSSION

### Scientific classification

Phylum: Chordata

Class: Teleostei

Order: Carangiformes

Family: Sphyraenidae

Genus: *Sphyraena*

Species: *Sphyraena forsteri*

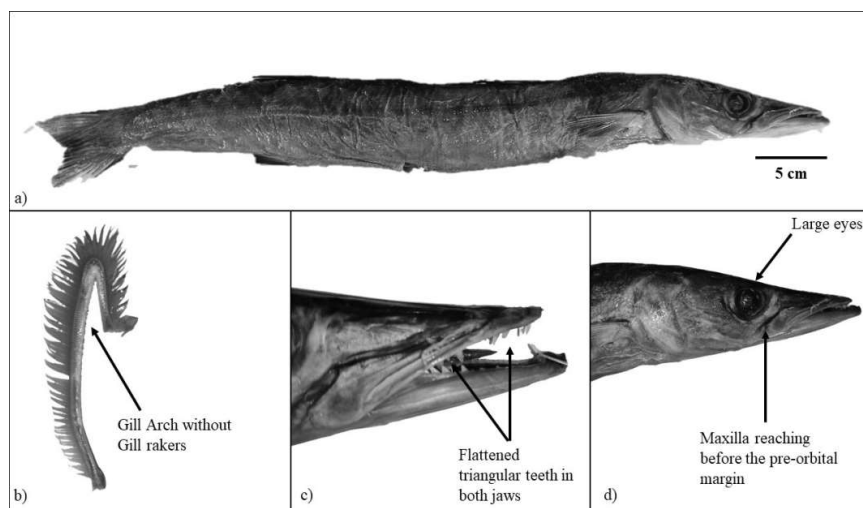
Total length of the species was 515 mm and 210 gm. Nineteen parameters were recorded on morphology of the fish (Table 1). The body is slender and long arrow-shaped with a pointy snout; the head of the species is larger ( $1/4^{\text{th}}$  of the total length) compared to other species of the family. The mouth is larger with flattened teeth in both jaws, the upper jaw has a few triangular teeth followed by an inner row of small teeth. One large canine tooth is present at the tip of the lower jaw, which fits into the upper jaw when the mouth is closed. Upper jaw slightly longer than lower jaw. The eyes are relatively large, comprising 14% of the head length. This

species has no gill rakers on the first gill arch, instead, there are rough platelets present, and the platelets on the lower arm have a few distinct spines. Origin of first spiny dorsal fin (D<sub>1</sub>VII) slightly behind the origin of pelvic fin (V 7). Pectoral fin (P 15) extended beyond pelvic fin. The second dorsal (D<sub>2</sub> 9) fin set slightly forward to the anal fin (A II 8).

**Table 1.** Morphometric parameters of *Sphyraena forsteri*

Parameter	Cm	% T.L.
Total length (T.L.)	51.5	100.00
Fork length	45.2	87.77
Standard length	41.2	80.00
Pre-anal length	31.4	60.97
Anal fin length	3.3	6.41
Pre-dorsal length	17.8	34.56
Dorsal length D1	4.1	7.96
Dorsal length D2	3.7	7.18
Inter dorsal length	8.0	15.53
Pre-pelvic length	15.4	29.90
Pelvic length	3.4	6.60
Prepectoral length	12.5	24.27
Pectoral length	4.6	8.93
Body depth	6.0	11.65
Pre-orbital length	6.3	12.23
Orbital length	1.8	3.50
Post-orbital length	4.7	9.13
Head length	12.7	24.66
Caudal peduncle length	7.3	14.17

\*All the morphometric parameters are calculated against the total length (T.L.) of specimen



**Fig. 1.** a) Lateral view of preserved specimen of *S. forsteri* b) First gill arch c) Lateral view of mouth and teeth d) Lateral view of head

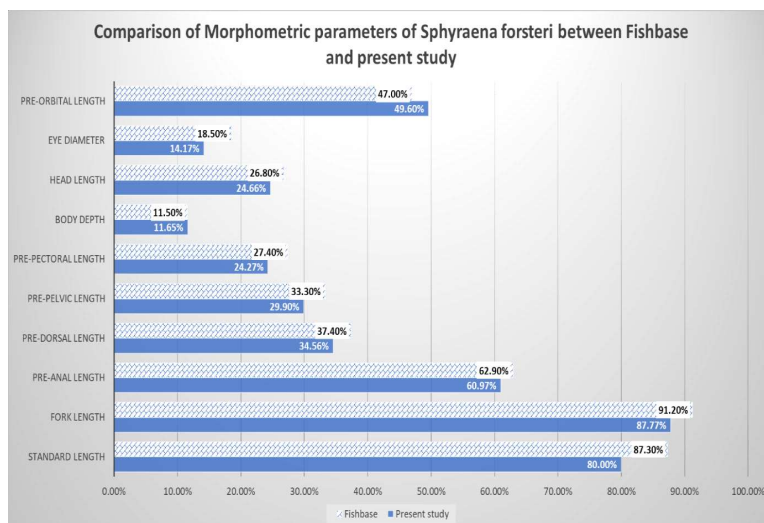


Fig. 2. Morphometric parameters of *S. forsteri* with those available on fishbase (Froese and Pauly 2024)

Numerous studies have examined the diversity of fish species across various harbours and fish landing sites in Gujarat. Notable investigations include by Brahmane et al. (2014), Katira (2017) and Parmar et al. (2022) in Sikka (northwestern Gujarat) and its vicinity; Sidat et al. (2021) along the Mandvi coast and the Gulf of Kutch; and Singh (2021) in Sutrapada. Additionally, Joshi et al. (2015), Sikotariya et al. (2018), and Solanki et al. (2020) have focused on fish diversity in Veraval Harbour, the fish market, and Okha Port, respectively. Despite the extensive efforts of these researchers, none have recorded the presence of *Sphyraena forsteri* anywhere in Gujarat. Furthermore Borichangar et al. (2022) at Dholai fishing harbour also did not document this species in southern Gujarat. This study provides the first confirmed record of *S. forsteri* from Dholai fishing harbour, filling a significant gap in the understanding of the region's ichthyofaunal diversity. The morphometric analysis conducted in this study was compared with data from FishBase (Froese and Pauly, 2024) (Fig. 2). The morphometric parameters of the specimen were evaluated against established identification keys, providing conclusive evidence for the identification of the species.

## CONCLUSION

The study confirm the presence of *Sphyraena forsteri* at the Dholai fishing harbour, marking its first geographical record from the southwest coast of Gujarat. The absence of this species in previously documented checklists highlights the significance of this discovery.

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Received 22 September, 2024; Accepted 22 December, 2024



# Traditional Antihelminthic Potential of Plants of Plampur Region of Lower Foothills of Shivalik Range of Himachal Pradesh

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**Abstract:** Traditional medicine has relied heavily on wild herbs, shrubs, and trees especially when treating helminthic illnesses. Various wild plants are utilized for their antihelminthic properties, offering natural and accessible alternatives to synthetic drugs. Among these are *Cordia dichotoma*, *Embllica officinale* (Indian gooseberry) and *Morus alba*. These plants are home to bioactive substances like tannins, flavonoids, alkaloids, and saponins, which exhibit potent antihelminthic activities. Traditional preparation methods include consuming raw extracts, powders, decoctions, and infusions. Modern scientific research supports these traditional uses, highlighting the efficacy of these plants in disrupting the metabolism of parasitic worms, altering their membrane permeability, and stimulating the host's immune response. The use of wild plants not only provides a sustainable and cost-effective treatment option but also preserves indigenous knowledge and promotes biodiversity. Despite the promising potential, there is a need for standardized dosages and comprehensive clinical trials to ensure safety and efficacy. The integration of traditional knowledge with modern science could enhance the management of helminthic infections, particularly in resource-limited settings.

**Keywords:** Antihelminthic, Traditional uses, Traditional knowledge of Wild plants

Globally, plants have long been used for medicinal purposes. These herbal remedies are often viewed as having lower toxicity and being gentler than pharmaceutical drugs, while also serving as a valuable source of bioactive compounds. In many developing countries, where modern medical systems may be limited, medicinal plants continue to be relied upon as a primary source of healthcare (Kaci et al., 2022). Himachal Pradesh, with a geographical area of 55,673 km<sup>2</sup>, forms part of the Trans and Northwest Himalayas, renowned for its unique biodiversity, with forests covering 66.45% of the state's total area, including 59.3% protected and 3.41% reserve forests (Kumar and Sharma 2016). Within this rich ecological landscape, the Palampur region in Kangra District, located in the lower foothills of the Shivalik range, supports a diverse ecosystem that has been sustainably utilized by local communities for generations (Singh et al., 2021). These regions are especially rich in plants with medicinal potential, including antihelminthic species (Rajeswari 2014). As the majority of the population in Palampur Tehsil resides in rural areas, the prevalence of unhygienic and unhealthy lifestyles contributes to the infiltration of parasitic worms into their bodies, leading to various diseases such as helminthiasis. Helminthiasis is an infection caused by parasitic roundworms in the intestines (Kumar et al., 2015). The two main groups of worms to which these species belong are nematodes (roundworms) and platyhelminths (flatworms), which include both cestodes (tapeworms) and trematodes (flukes) (Mali and Mehta 2008).

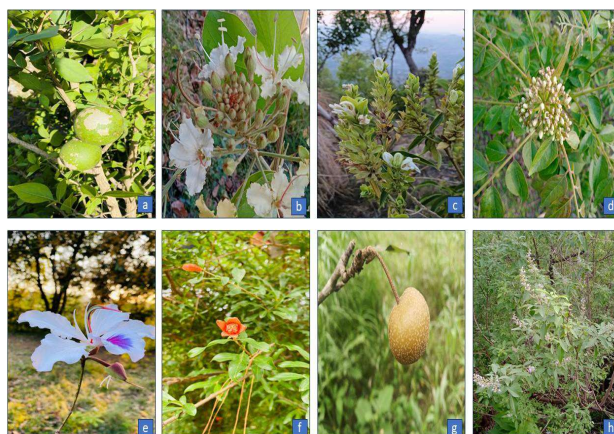
The significant worldwide health concern, helminthiasis is especially frequent in tropical and subtropical areas where sanitation and hygiene standards may be low (Busari et al., 2024, King 2019). The consumption of contaminated food or water, contact with contaminated soil, or bites from infected vectors (such as mosquitoes or flies) are the three main ways that helminthiasis is transmitted (Tahseen 2018). Direct contact with animals or people who are infected can also result in infection. A significant section of the global population suffers from helminthic diseases, which are among the most prevalent illnesses in humans (Tariq et al., 2009). Traditional healers in Palampur and surrounding areas have traditionally used a range of wild plants that have been shown to be beneficial in treating parasitic worm infestations when it comes to treating helminthic illnesses. These healers play a crucial role in managing, conserving, and ensuring the sustainable use of medicinal plants, a knowledge passed down through generations from their ancestors (Gangadhar 2022). The rich flora of this region includes numerous species reported to possess antihelminthic properties (Rajeswari 2014). This traditional knowledge represents a valuable resource for the potential development of new antihelminthic treatments, highlighting the importance of preserving both the biodiversity of the region and the associated Indigenous knowledge (Kumari et al., 2019). The indigenous population of this region has preserved and passed down extensive knowledge about medicinal plants through oral traditions and cultural

practices. This wisdom encompasses various aspects of plant use, harvesting techniques, and preparation methods for therapeutic applications. Some commonly used plants include *Punica granatum* and *Embllica officinalis*. *P. granatum* contain bioactive substances with potential anthelmintic properties, such as cucurbiti. The bark, roots, and fruit rind of this plant have all been traditionally employed for their therapeutic qualities, which include anthelmintic action (Kiran et al., 2024). Pomegranate compounds including punicalagin and ellagic acid have been investigated for their antiparasitic properties. *E. officinalis* L. is also known by another name, amla, the Indian gooseberry is a highly valued medicinal herb in Ayurveda (Yadav and Singh 2023). It is used in traditional formulations to treat intestinal parasites and is thought to have anthelmintic qualities. These are just a few of the many antihelminthic herbs that are traditionally observed in Palampur (Rawal et al., 2023). In this study, the traditional antihelminthic activities of plants were investigated. Both wild edible plant species and cultivated plants were selected for this purpose. Their potential to combat helminthic infections was explored through this research. It is also necessary to record customary knowledge regarding the utilization of plant resources of this region as folk remedies for various health problems including helminth-related problems for the benefit of future generations.

### MATERIAL AND METHODS

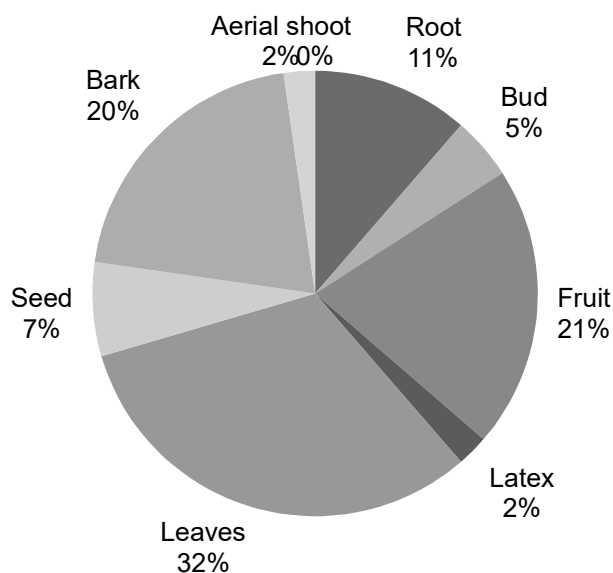
The study included extensive field surveys across various villages in Kangra District, Himachal Pradesh, conducted in different seasons. Researchers used interactive interview

methods, including questionnaire surveys, informal gatherings, field observations, and group discussions. They interviewed knowledgeable individuals aged 35 to 65, including men, women, youths, and elders, mostly involved in agriculture and horticulture. Semi-structured questionnaires were used to collect details on the traditional use of medicinal plants. Initially, plant specimens were shown to villagers and local healers to gather information on their therapeutic uses. Detailed information was then collected about the plants, the parts used, and how they were utilized. The documented information was analyzed based on several parameters. Fresh plant samples were collected and identified using



a. *Aegle marmelos* (L.) Correa b. *Bauhinia vahlii* Wight & Arn. c. *Justicia adhatoda* L. d. *Murraya koenigii* (L.) Spreng. e. *Bauhinia variegata* Linn. f. *Punica granatum* L. g. *Pyrus pashia* Buch.-Ham. ex D. Don. h. *Vitex negundo*

**Fig. 2.** Wild plants of Palampur region of lower foothills of Shivalik range of Himachal Pradesh



**Fig. 3.** Percentage of different plant parts used as Antihelminths

**Table 1.** Traditional anthelmintic potential of some medicinal plants that are used by local and rural people in Palampur region of lower foothills of Shivalik range of Himachal Pradesh

Botanical name	Family	Local name	Part used	Uses
<i>Asparagus racemosus</i> Willd.	Asparagaceae	Sansarpali, Sansfain, Shatavri	Root	The roots of this plant are dried and ground into a fine powder. 3-6 grams of this powder mixed with lukewarm is taken orally once or twice daily, usually mixed with water, honey, or milk, to expel tapeworm infections.
<i>Aegle marmelos</i> (L.) Correa	Rutaceae	Bill, bail patri	Fruit	5-10g powder of unripe fruit is taken with lukewarm water on an empty stomach for 4-5 days to cure intestinal worms.
<i>Ageratum conyzoides</i> L.	Asteraceae	Phulanu	Bud	The 10-15 floral buds of this plant are dried and crushed to make a fine powder, and 6-8 grams of this powder is taken on an empty stomach with lukewarm water daily for three days to expel various types of worms for example flatworms and liver fluke from the body.
<i>Aloe barbadensis</i> Mill.	Liliaceae	Kawaraya, Ghritkumari	Leaf	5-10ml of juice from its fresh leaves are used to drink daily to kill parasitic intestinal worms like threadworms.
<i>Argemone mexicana</i> L.	Papaveraceae	Bharbhand, Kanduri	Root	5-10ml decoction of its roots is taken daily three times to eliminate the intestinal worms. The grind powder of its root is taken with juice extracted from the onion bulb to expel the pinworms immediately.
<i>Bauhinia vahlii</i> Wight & Arn.	Fabaceae	Torr	Bark	3-4g of dried powder of its stem bark is taken with lukewarm water twice daily for 4-5 days to expel and kill the larvae and adults of intestinal parasitic worms, mainly <i>Ascaridia galli</i> .
<i>Bauhinia variegata</i> Linn.	Fabaceae	Karyala, Kachnar	Bark, Bud	3g dried bud powder of this plant is taken two times a day with lukewarm water to expel the intestinal worms. 5-10ml decoction of its bark is taken empty stomach for three days to expel the liver fluke and flatworms.
<i>Cordia dichotoma</i> G.Forst	Boraginaceae	Lasuda	Leaf, Fruit	3-5ml juice from fresh leaves is used to drink daily for one week to expel the parasitic intestinal worms. 50-100 grams of roasted fruits eaten for one week to the expulsion of threadworms.
<i>Chenopodium album</i> L.	Chenopodiaceae	Bathu	Leaf, Seed	10-15ml of infusions made from leaves and seeds boiling together are used to drink daily two times a day for one week to treat intestinal worms mainly hookworm and pinworm.
<i>Cannabis sativa</i> Linn.	Cannabinaceae	Bhang	Leaf	15-20 ml decoction of leaves is taken three times a day for three to four days to get rid of intestinal threadworm.
<i>Emblica officinalis</i> Gaertn	Euphorbiaceae	Amla	Fruit	The dried fruit is ground into a fine powder. Traditionally, the powder is consumed in small doses, often mixed with water, honey, or ghee. The regular consumption of amla powder is believed to help in expelling intestinal worms like hookworms due to its potent anthelmintic properties.
<i>Ficus palmata</i> L.	Moraceae	Anjir	Fruit	4-5 dried fruits are often consumed directly or soaked in water overnight. Eating this soaked dried fruit in the morning on an empty stomach is believed to help expel intestinal worms.
<i>Ficus religiosa</i> L.	Moraceae	Peepal	Bark, Latex	Decoction prepared from its bark is beneficial for killing and paralyzing various kinds of intestinal worms mainly <i>Ascaris</i> . The latex of this plant possesses vermifuge properties and is used to expel various kinds of worms.
<i>Justicia adhatoda</i> L.	Acanthaceae	Basuti, Vaska	Leaf and Bark	20-25 ml of juice extracted from the leaves and bark is drunk once a day for one week to eliminate threadworm and hookworm.
<i>Mallotus philippensis</i> (Lam.) Müll.Arg.	Euphorbiaceae	Kamala	Seed, Fruit, Bark, Leaf	The decoction prepared from its dried seed, leaves, fruit, and bark with water which is taken 3 times a day for 3 days is used to kill and expel the tapeworms.
<i>Melia azedarach</i> L.	Meliaceae	Drek	Leaf, Seed	Decoction prepared from its leaves is taken daily once a day for 2-3 weeks to expel parasitic worms. Dried seed powder with lukewarm water is taken two times daily for half a week to treat intestinal worms.
<i>Morus alba</i> L.	Moraceae	Toot	Root, Fruit	5-10ml decoction prepared from the root is used to drink after a meal for one week to expel the stomach worms. 30-50 grams of fresh fruits have been eaten daily to cure the intestinal worms.

Cont...

**Table 1.** Traditional anthelmintic potential of some medicinal plants that are used by local and rural people in Palampur region of lower foothills of Shivalik range of Himachal Pradesh

Botanical name	Family	Local name	Part used	Uses
<i>Murraya koenigii</i> (L.) Spreng.	Rutaceae	Kadi patta	Leaf	5 ml decoction of its leaves is taken two times a day for three days to kill and paralyze the intestinal parasites in humans mainly liver fluke and pinworm and gastrointestinal nematode parasites in sheep.
<i>Nyctanthes arbor-tristis</i> Linn.	Oleaceae	Raat ki Chameli, Haar Shringaar	Leaf, Bark	The extract from its fresh leaves mixed with salt is taken to an empty stomach in the morning for 3 days to eliminate intestinal threadworms. Dried powder of leaves is used with water in cases of liver fluke intestinal worms. Bark extract is also used for expelling roundworms and intestinal worms due to the presence of tannin.
<i>Oxalis corniculata</i> L.	Oxalidaceae	Malori	Leaves	10-15ml decoction made from the leaves is taken daily twice a day for 3-4 weeks to get rid of intestinal worms.
<i>Punica granatum</i> L.	Punicaceae	Dhadu, Dhadan	Bark, Fruit	The dried powder of fruit peel is taken with lukewarm water two times a day for one week killing and paralyzing the worms. The juice extract from the fruit is drunk daily to expel the hookworms.
<i>Pyrus pashia</i> Buch.-Ham. ex D. Don	Rosaceae	Kainth	Bark	3g dried bark churan is taken with lukewarm water and is useful against various kinds of parasitic worms like hookworms and threadworms.
<i>Rubus ellipticus</i> Sm.	Rosaceae	Aakhe	Root	Root decoction of about one teaspoon is given to the children once a day for 4-5 days to get rid of intestinal worms.
<i>Thevetia neriifolia</i> (Pers.) K.Schum.	Apocynaceae	Peeli Kaner	Leaf	5-8ml decoction of leaves of this plant is taken for a week, three times every day to expel the intestinal worms.
<i>Tamarindus indica</i> Linn.	Fabaceae	Imli	Fruit, Leaf	10-15 ml of juice from the fruit drink one time a day for 4-5 days helps expel the worms of the intestine. 5-10 ml of juice from the fresh leaves is drunk daily, two times a day, which helps paralyze and kill stomach worms.
<i>Terminalia chebula</i> Retz.	Combretaceae	Harad	Fruit, Bark	50 ml Juice extracted from its fruit pulp is taken twice daily to eliminate tapeworm and Ascaris. Churan prepared from its dried bark mixed with harad ( <i>Terminalia chebula</i> ) and amla ( <i>Embellica officinalis</i> ) is known as triphalachuran, which is good for digestive problems and possesses anthelmintic properties. So, this churan is used to expel the parasitic worms.
<i>Trifolium rapens</i> L.	Fabaceae	Khukhani	Aerial shoot	2ml decoction of its aerial shoots is taken three times a day for two days to kill and paralyze intestinal helminthic worms mainly tapeworms. This extract is also used to kill intestinal worms in sheep mainly liver fluke.
<i>Urtica dioica</i> L.	Urticaceae	Bichu-buti, Aan	Leaf, Root	Fresh or dried leaves are steeped in hot water to make tea. The tea is consumed regularly to help expel worms and improve overall gut health. Roots of the plant are boiled in water for a longer period to extract active compounds. The resulting decoction is drunk daily for a specified duration, usually a few weeks, to clear hookworm parasitic infections.
<i>Vitex negundo</i> L.	Verbenaceae	Bana	Leaf	3 ml decoction of its leaves is used to kill and paralyze the various kinds of threadworm and flatworm intestinal parasites within a day.

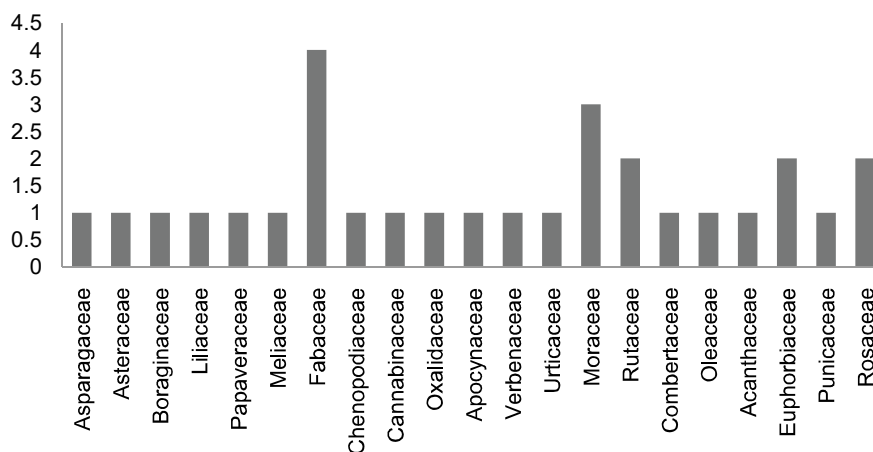
various botanical references, such as 'The Flora of British India' (Hooker 1885), 'Flora of Himachal Pradesh' (Chowdhery and Wadhwa 1984), and 'Flora of Mandi' (Singh 2018).

The current study provided details on wild plants that have anthelmintic properties. The names of the plants' families, local names, botanical names, parts used, and their traditional uses for helminthiasis are listed in alphabetical order, as shown in Table 1. The present study includes 29 plants belonging to 21 families. The predominant families are

Fabaceae with 4 plant species, Euphorbiaceae, Rosaceae, and Rutaceae with 2 plant species, and Moraceae with 3 plant species (Fig. 4).

Among the plant parts, leaves are the most commonly used, accounting for 32%, followed by fruits (21%) and bark (20%). Roots are utilized in 11% of the cases, while buds and seeds in 5 and 7percent (Fig 1). Latex and aerial shoots are the least commonly used, each accounting for 2%. This distribution highlights the predominance of leaves, fruits, and bark in traditional anthelmintic practices as shown in Figure





**Fig. 4.** Showing the predominant families of the plants in the study area having anthelmintic properties

3. The 3-4 g of powder of dried roots of the *Asparagus racemosus* plant mixed with lukewarm water is taken orally once or twice daily, usually mixed with water, honey, or milk, to expel tapeworm infections. Similar results have been shown by Soren and Yadav (2021). The 5-10 ml decoction prepared from the root of *Morus alba* is used to drink after a meal for one week to expel the stomach worms. Devi et al. (2013) also mentioned similar results. The 4-5 dried fruits of *Ficus palmata* are often consumed directly or soaked in water overnight. Eating this soaked dried fruit in the morning on an empty stomach is believed to help expel intestinal worms. Badgujar et al. (2014) observed similar trend.

### CONCLUSION

The exploration of wild plants from the Palampur region of Himachal Pradesh for their antihelminthic properties shows the significant potential of traditional medicinal knowledge in addressing parasitic infections. This study has documented various plants traditionally used by local communities, such as *Cordia dichotoma*, *Emblica officinale*, and, which exhibit potent antihelminthic activities. The traditional methods of preparation and administration, including raw extracts, powders, decoctions, and infusions, provide sustainable and cost-effective alternatives to synthetic drugs. These practices not only offer immediate health benefits but also help preserve indigenous knowledge and promote biodiversity. The detailed documentation of these plants' uses, parts utilized, and preparation methods highlights the rich ethnobotanical heritage of the Palampur region. Integrating traditional knowledge with modern scientific research could lead to the development of new, natural antihelminthic drugs, particularly beneficial in resource-limited settings where synthetic drugs may be inaccessible. As a vital resource in the fight against helminthic illnesses,

the outcome of the research emphasizes the significance of wild plants. It supports more research into conventional medicine along with contemporary scientific verification to improve the treatment of parasitic illnesses and advance global health solutions.

### AUTHOR'S CONTRIBUTION

Devi R: Prepared the original draft of the manuscript; Kumar N: edited the manuscript; Saurav, Saklani S, and Kumari N Reviewed the manuscript and confirmed the authenticity of all the data. All authors have read and approved the final manuscript.

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# Conservation Status, Diversity and Utilization Pattern of Threatened Ethno-Medicinal Plants of Betalghat Region, Kumaun Himalaya

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**Abstract:** Himalaya is one of the repository of medicinal plants. The study was conducted for the documentation of threatened ethno-medicinal plants from Betalghat region, Kumaun Himalaya. Betalghat is a block of Nainital district located at the bank of river Kosi. The present study records 20 species of threatened plants belonging to 16 families and 18 genera. Out of which 3 species were trees, 4 shrubs, and 13 herbs. Caprifoliaceae, Lamiaceae and Orchidaceae were the dominant families. Out of 20 species, 9 species were near threatened, 6 species were least concern, 4 species were vulnerable and 1 species were endangered. Local Name, botanical Name, family, habit, altitudes (m), habitat and utilization pattern were provided for each species. Therefore, there is an urgent need for conservation steps to be taken up along with promotion of conservation of threatened medicinal plants.

**Keywords:** Threatened, Medicinal plants, Status, Betalghat region, Kumaun Himalaya

The erosion of biodiversity is an issue of global concern. In ecosystems building blocks are disappearing, one by one. The number of species, which are going threatened, is increasing gradually. The numbers of threatened plants are approximately 8457, out of which 247 plants are in various biodiversity hotspots of India (IUCN 2010). The use of plants by the local inhabitants of these regions for various purposes, i.e., as medicine, as edible, fodder, fuel, timber, in making agriculture tools, cultural and various others has occurred since time immemorial. The resurgence of public interest in plant-based medicine related to the rapid expansion of the pharmaceutical industry prompted overexploitation that threatened many useful medicinal plants (Kumari et al., 2012). Further, the level of threat to the natural population of medicinal plants has increased. More than 90% of India's raw plant products are sourced from natural environments for different herbal industries (Dhar et al., 2002). In the coming 20 to 30 years, 25 percent of all biodiversity could become extinct. The habitat fragmentation by an excessive clearing of native vegetation poses a significant threat to floral and faunal biodiversity (Ford et al., 2009).

The regular exploitation of various medicinal flora from the wild habitat and substantial loss of their habitats during the last 15 years have resulted in a population loss of various valuable medicinal plant species over the years. The main threats to medicinal plants influence any biodiversity that peoples utilize. The debility of traditional laws regulating natural wealth utilization is among the main reasons for threatening the wild medicinal plant species (Kala 2005, Kala and Ratajc 2012). Belt et al. (2003) observed that these

traditional laws have regularly proved to be simply damaged by modern socio-economic forces. Various possible reasons of a rarity in medicinal plant species, such as climatic changes, the explosion of the human population, fragmentation and degradation of population, habitat alteration and specificity, genetic drift, the introduction of non-natives, heavy grazing, narrow range of distribution and land-use disturbances (Kala 2005, Kala and Ratajc 2012).

However, the insufficient available data on the threatened species in nature has restricted their categorization to a few species based on herbarium collection and by consultation by a couple of specialists (Kala 2005, Kala and Ratajc 2012). The problems in assessing the plant species diversity are increased in the Himalayan region, in respect of high altitude areas because of inhospitable climatic conditions, rough and detachment of the terrain, and short life cycle of plants (Kala and Ratajc 2012). The indigenous communities and commercial herb gatherers also raid these same landscapes to collect valuable medicinal plants. Therefore, the predictable population density of categorized threatened medicinal plants is not exact. It varies the region that never and scarcely undergone any collection visits of such rare medicinal plant species (Kala 2005, Kala and Ratajc 2012).

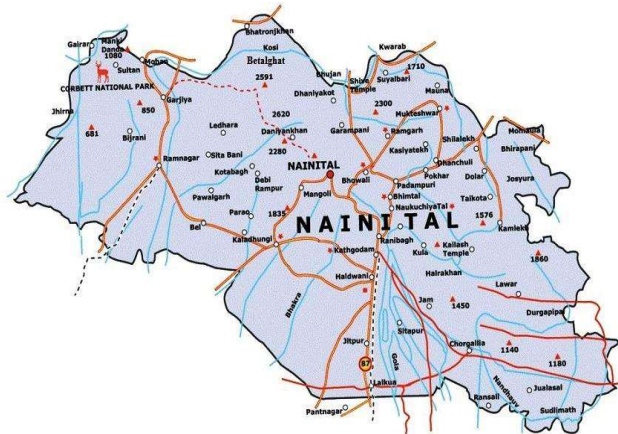
For short or long term management planning and area-specific threat categorization of medicinal plant species is important (Singh et al., 2009). The availability of critically endangered, endangered, and vulnerable medicinal plants indicates high anthropogenic pressure. If continuous overexploitation and habitat degradation of these species, they may disappear from the area shortly (Singh et al., 2009,

Kumari et al., 2012, Sharma et al., 2014). There is an urgent need of the establishment and conservation of medicinal plants nurseries and herbal gardens and ensuring the availability of quality planting material for large-scale cultivation, through educational and awareness programs (Singh et al., 2009, Kumari et al., 2012, Sharma et al., 2014). The present study represents such an effort in the study area, using the information on different attributes.

## MATERIAL AND METHODS

**Study area:** The Betalghat block lies between 29°32'45"-29°29'36"N latitudes and 79°14'43"-79°24'24"E longitudes. Betalghat is a block of Nainital district located at the bank of river Kosi (Fig. 1). It is situated in the northern part of the district Nainital, Kumaun Himalaya.

**Data collection and analysis:** The field surveys were carried out during the year 2022-2024. Specimens of all the plants were collected and identified with the help of relevant floras and herbaria (Gaur 1999, Joshi et al., 2018). The specimens collected from the field were deposited in the herbarium division of the Department of Botany, D.S.B. Campus, Kumaun University, Nainital. Using six attributes (i.e., habitat preference, distribution range, population size, use pattern, extraction trend, native and endemic species), the threat status was identified and also, categorization of these species is done as Least Concern, Near Threatened, Rare, Vulnerable, Endangered and Critically Endangered



(Source: <http://www.uttaranchal.org.uk>)

**Fig. 1.** Map of the study area

following (Samant et al., 1998, Dhar et al., 2002, Kala 2005, Singh et al., 2009, Kala and Ratajc 2012). Species with scores <46 were identified as least concern, 46-50 as near threatened, 51-55 as vulnerable, 56-60 as endangered, and >60 as critically endangered (Table 1).

## RESULTS AND DISCUSSION

**Diversity of threatened medicinal plants:** The present study recorded 20 species of threatened plants belonging to 16 families and 18 genera. Out of which 3 species were trees, 4 shrubs, and 13 herbs (Table 2, Plate 1). The families were Caprifoliaceae, Lamiaceae, and Orchidaceae, having two species and Apiaceae, Asparagaceae, Bignoniaceae, Celastraceae, Colchicaceae, Costaceae, Dioscoreaceae, Euphorbiaceae, Hypoxidaceae, Pittosporaceae, Ranunculaceae, Rutaceae, Saxifragaceae, and Ulmaceae having single species were identified. Along an altitudinal gradient, altitudinal zone 1001-1300m showed the maximum diversity (i.e., 12 spp.), followed by the zone 700-1000 m (11 spp.), 1601-1800 m (10 spp.), and 1301-1600 m (8 spp.) respectively. Overlapping within different altitudinal zones is noted in most of the cases.

**Status of threatened medicinal plants:** In the present study, 6 species (*Bergenia ciliata* (Haworth) Sternb., *Celastrus paniculatus* Will., *Curculigo orchoides* Gaertn., *Thalictrum foliolosum* DC., *Valeriana wallichii* DC. and *Zanthoxylum armatum* DC.) with scores 40-44 were considered as least concerned category, 9 species (*Baliospermum montanum* (Willd.) Muell.-Arg., *Clerodendrum serratum* L., *Coleus barbatus* (Andr.) Benth, *Costus speciosus* (Koenig ex Retz.) Sm., *Dioscorea deltoidea* Wall. ex Griseb., *Drimia indica* (Roxb.) Jessop., *Malaxis acuminata* D. Don, *Ulmus wallichiana* Planch, and *Valeriana hardwickii* Wall.) with score 48 were found under near threatened category, and 4 species (*Gloriosa superba* L., *Habenaria intermedia* D. Don, *Heracleum lanatum* Michaux and *Oroxylum indicum* L.) with score 52 were found under vulnerable category, and 1 species (*Pittosporum eriocarpum* Royle.) with score 56 were under endangered category (Table 3).

The Himalayan region has rich biological diversity that is now under threat from rapidly expanding human populations, habitat destruction, and concomitant environmental

**Table 1.** Different criteria for threat categorization of the species

Habitat	Distribution	Population (Individual/ location)	Use pattern	Extraction trend	Native and endemic	Score
One	<500	250 Individual/up to 2 location	4 and >4	Commercial	Native and endemic	10
2-3	500-1000	250-1000 Individual/3-5 location	2-3	Self-use	Native/Endemic	6
>3	>1000	1000 Individual/>5 location	One	No use	Non-native	2

**Table 2.** Diversity and utilization pattern of threatened ethno-medicinal plants of Betalghat Region, District Nainital (Kumaun Himalaya)

Local name	Botanical name	Family	Habit	Altitudes (m)	Habitat	Utilization pattern
Danti	<i>Baliospermum montanum</i> (Willd.) Muell.-Arg.	Euphorbiaceae	Sh	700-1100	Forest openings, edges, and wastelands	Arthritis, bronchitis diseases, constipation, jaundice, and skin disease.
Silphora	<i>Bergenia ciliata</i> (Haworth) Sternb.	Saxifragaceae	H	1400-1800	Shady and moist rocks	Asthma, boils, cough, diarrhea, fever, gall bladder, and kidney stones.
Mal-kangani	<i>Celastrus paniculatus</i> Willd.	Celastraceae	Sh	700-1100	Open places, rocky substrate	Arthritis, cough, diarrhea, dysentery, gout, headache, itching, piles, and wounds.
Bharangi	<i>Clerodendrum serratum</i> (L.) Monn.	Lamiaceae	Sh	700-1100	Roadside, open places	Asthma, bronchitis, cough, headache, fever, and skin diseases.
Hiwain	<i>Coleus barbatus</i> (Andr.) Benth	Lamiaceae	H	1100-1500	Rocky substrate	Constipation, low blood pressure, and intestinal worm infection.
Keva	<i>Costus speciosus</i> (J. Koenig) Sm.	Costaceae	H	700-1100	Shady forest slopes	Arthritis, cold, cough, and stomach-ache.
Kali Musli	<i>Curculigo orchioides</i> Gaertn.	Hypoxidaceae	H	700-1600	Open grassy localities, undergrowth in moist shady areas,	Asthma, cough, cuts and wounds, diarrhoea, jaundice, Insect or scorpion bite, itching piles, skin diseases, and urinary disorders.
Jangli Gethi	<i>Dioscorea deltoidea</i> Wall. ex Griseb.	Dioscoreaceae	H	1600-1800	Open places	Dysentery, fever, jaundice, and skin diseases.
Ban Pyaj	<i>Drimia indica</i> (Roxb.) Jessop.	Asparagaceae	H	700-1100	Open exposed, grassy slopes	Joints, pain, and arthritis.
Kalihari	<i>Gloriosa superba</i> L.	Colchicaceae	H	700-1100	Along forest margins	Fever, gonorrhoea, insect or scorpion stings, leprosy intermittent, piles, painful delivery, rheumatism, skin diseases, and tumors.
Vridhi	<i>Habenaria intermedia</i> D. Don	Orchidaceae	H	1600-1800	Open grassland, grassy slopes	Asthma, health tonic and skin diseases.
Kakriya	<i>Heracleum lanatum</i> Michaux	Apiaceae	H	1600-1800	Shady forests edges and open fields	Arthritis, leucoderma and toothaches.
Jivak	<i>Malaxis acuminata</i> D. Don	Orchidaceae	H	1500-1800	Moist shady places	Fever, bronchitis, general debility, and weakness.
Syonake	<i>Oroxylum indicum</i> (L.) Kurz	Bignoniaceae	T	700-1000	Open places	Diarrhea, dysentery, fever, indigestion, insect and scorpion stings, stomach-ache and urinary disorders.
Agni	<i>Pittosporum eriocarpum</i> Royle.	Pittosporaceae	T	900-1300	Banj-oak and miscellaneous forests	Bronchitis and rheumatic swellings.
Mamiri	<i>Thalictrum foliolosum</i> DC.	Ranunculaceae	H	900-1800	Open hill slopes	Conjunctivitis boils, and jaundice.
Chamar-Mua	<i>Ulmus wallichiana</i> Planch	Ulmaceae	T	1600-1800	Banj-oak forests	Bone fracture.
Sameo	<i>Valeriana hardwickii</i> Wall.	Caprifoliaceae	H	1100-1800	Moist shady slopes	Diarrhoea, fever, and urinary disorders.
Sameo	<i>Valeriana wallichii</i> DC.	Caprifoliaceae	H	1400-1800	Moist places, forest floors	Cholera and urinary disorders.
Timur	<i>Zanthoxylum armatum</i> DC.	Rutaceae	Sh	800-1800	Open scrub jungles and grazing places	Mouth wash, toothache, headache, and asthma.

**Abbreviations used-** H: Herb; Sh: Shrub; T: Tree

degradation occurring at a fast pace. At the regional, national and global levels various attempts have been made to identify threatened species, including medicinal plants, using different attributes such as biogeographical range, habitat preference, population size and utilization pattern (Kala and Ratajc 2012, Kumari et al., 2012, Chauhan et al., 2014, Lone et al., 2014, Manikandan and Srivastava 2015, Singh et al., 2017, Arya et al., 2018, Jeph and Khan 2019, Bhatt et al., 2020, Chandra et al., 2021, Rawal and Tewari 2022 ).

The total of 121 species of vascular plants, including 17 species of medicinal plants from IHR, have been recorded in the Red Data Book of Indian Plants (Nayar and Shastri, 1987, 1988, 1990). It is therefore evident from the present investigation that all the documented threatened plants of present study belong to different threat categories according to the Red Data Book of Indian Plants (Nayar and Sastry 1987, 1988, 1990), CAMP (Conservation Assessment and Management Plan 1998, 2003) Workshop, other existing literature (Kumari et al., 2012, Srivastava and Singh 2005, Bhatt et al., 2020). At regional and global levels, threat categorization has also been done in which *Baliospermum montanum* (Willd.) Muell.-Arg. and *Celastrus paniculatus* Will. are under the near-threatened category, *Bergenia ciliata*

(Haworth) Sternb., *Clerodendrum serratum* L., *Curculigo orchioides* Gaertn, and *Thalictrum foliolosum* DC. under a vulnerable category, *Gloriosa superba* L. and endangered categories and *Dioscorea deltoidea* Wall. ex Griseb., *Valeriana wallichii* DC. under critically rare categories (CAMP 1998). Srivastava and Singh (2005) reported 60 species of threatened medicinal plants from Uttarakhand, Joshi et al. (2010) reported 33 species of threatened medicinal plants from Alpine region of Uttarakhand, Kumari et al (2012) reported 21 species of threatened ethno-medicinal from district Almora, Kumaun Himalaya, Manikandan and Srivastava (2015) reported 30 species of threatened plants from Govind Pashu Vihar Wildlife Sanctuary, Western Himalaya, Rai et al (2017) reported 18 species of threatened plants Kedarnath Wildlife Sanctuary, Garhwal Himalaya, Uttarakhand, Singh et al. (2017) reported 29 species of threatened plants from Jakholi block of district Rudraprayag, Western Himalaya, Jeph and Khan (2019) reported 39 species of threatened plants from Jhunjhunu District, Rajasthan, Bhatt et al. (2020) reported 50 species of threatened plants from Kumaun Himalaya, Chandra et al. (2021) reported 14 species of threatened medicinal and aromatic plants from Western Himalaya and Rawal and

**Table 3.** Status and threats of threatened ethno-medicinal plants of Betalghat Region, Nainital District (Kumaun Himalaya)

Botanical name	Family	Observed status	CAMP status (1998, 2003)	Threats
<i>Baliospermum montanum</i> (Willd.) Muell.-Arg.	Euphorbiaceae	NT	NT	G, HD
<i>Bergenia ciliata</i> (Haworth) Sternb.	Saxifragaceae	LC	VU	OE, HD
<i>Celastrus paniculatus</i> Will.	Celastraceae	LC	VU	HD
<i>Clerodendrum serratum</i> L.	Lamiaceae	NT	VU	HD
<i>Coleus barbatus</i> (Andr.) Benth	Lamiaceae	NT	VU	HD
<i>Costus speciosus</i> (Koenig ex Retz.) Sm.	Costaceae	NT	NT	HD, G
<i>Curculigo orchioides</i> Gaertn.	Hypoxidaceae	LC	VU	OE, G
<i>Dioscorea deltoidea</i> Wall. ex Griseb.	Dioscoreaceae	NT	EN	HD
<i>Drimia indica</i> (Roxb.) Jessop.	Asparagaceae	NT	VU	HD, OE
<i>Gloriosa superba</i> L.	Colchicaceae	VU	VU	HD
<i>Habenaria intermedia</i> D. Don	Orchidaceae	VU	EN	HD
<i>Heracleum lanatum</i> Michaux	Apiaceae	VU	EN	HD, OE
<i>Malaxis acuminata</i> D. Don	Orchidaceae	NT	VU	HD, OE
<i>Oroxylum indicum</i> L.	Bignoniaceae	VU	VU	HD, OE
<i>Pittosporum eriocarpum</i> Royle.	Pittosporaceae	EN	EN	HD
<i>Thalictrum foliolosum</i> DC.	Ranunculaceae	LC	VU	OE
<i>Ulmus wallichiana</i> Planch	Ulmaceae	NT	VU	HD, OE
<i>Valeriana hardwickii</i> Wall.	Caprifoliaceae	NT	VU	G, HD
<i>Valeriana wallichii</i> DC.	Caprifoliaceae	LC	VU	OE
<i>Zanthoxylum armatum</i> DC.	Rutaceae	LC	VU	HD, OE

**Abbreviations Used-** EN: Endangered; VU: Vulnerable; NT: Near Threatened; LC: Least Concern; OE: Over Exploitation; HD: Habitat Degradation; G: Grazing; CAMP: Conservation Assessment and Management Plan



*Baliospermum montanum*



*Bergenia ciliata*



*Celastrus paniculatus*



*Clerodendrum serratum*



*Coleus barbatus*



*Costus speciosus*



*Curculigo orchioidea*



*Dioscorea deltoidea*



*Drimia indica*



*Gloriosa superba*



*Habenaria intermedia*



*Heracleum lanatum*



*Malaxis acuminata*



*Oroxylum indicum*



*Pittosporum eriocarpum*



*Thalictrum foliolosum*



*Valeriana wallichii*



*Zanthoxylum armatum*

**Plate 1.** Threatened plants of Betalghat Region, District Nainital(Kumaun Himalaya)

Tewari (2022) reported 16 species of threatened plants from Kedarnath Wildlife Sanctuary, Garhwal Himalaya, Uttarakhand. The present study reports 20 species of threatened plants belonging to 17 families and 19 genera. Out of which 3 species were trees, 4 shrubs, and 13 herbs. It is observed that out of 20 species, 9 species were near threatened, 6 species were least concern, 4 species were vulnerable and 1 species were endangered.

### CONCLUSION

The indicate that 20 species of threatened plants belonging to 17 families and 19 genera. The six species are considered as least concerned category, 9 species under near threatened category, 4 under vulnerable category, and 1 species under endangered category in the study area. Documentation of these threatened medicinal plants may provide basic information for conservation and sustainable development of the Himalayan region.

### AUTHOR CONTRIBUTIONS

Naveen Ch. Pandey, Geetanjali Upadhyay, Disha Upreti, Anand Kumar and Vasundhra Lodhiyal: formal analysis, investigation, methodology, writing-original draft. Lalit M. Tewari: supervision, visualization, conceptualization, review and editing.

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# Soil-Site Suitability Assessment of Horticultural Crops Grown in the Ganjigatti Sub-watershed of Karnataka

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**Abstract:** The initial step in planning future agricultural land use is to establish a connection between soil characteristics and crop requirements. The objective of this study is to assess the limitations and potential of soils within the Ganjigatti sub-watershed in Karnataka and to determine their suitability for major horticultural crops grown in that sub-watershed. During the land resource inventory of the study area, twenty-one soil series were identified and mapped. Using criteria such as texture, depth, slope, erosion, gravel content and stoniness, twenty-one soil series were categorized into sixty-one mapping units. These soils were evaluated for their suitability for major horticultural crops, including chilli, tomato, rose and jasmine, in the Ganjigatti sub-watershed, located in the hilly zone (Zone 9) of Karnataka, with a focus on sustainable land use planning. The sub-watershed had predominantly moderate (S2) and marginally (S3) suitability for various crops. For chilli production, 2181 ha (50.46%) and 1571 ha (36.33%) were moderately and marginally suitable, respectively, but faced limitations such as length of the growing period, soil drainage, texture, depth, gravel content and slope. Similarly, for tomato production, 1294 ha (29.92%) and 2460 ha (56.88%) were moderately and marginally suitable, respectively, with similar limitations. For rose production, 899 ha (20.79%) and 1337 ha (30.92%) were moderately and marginally suitable, respectively, with issues related to soil drainage, texture, depth, gravel content and calcium carbonate (CaCO<sub>3</sub>) levels. Approximately 36.43% of TGA were not suitable for rose production due to severe depth limitations. For jasmine production, 30.30% and 56.64% of TGA were moderately and marginally suitable, respectively, but faced limitations concerning average annual temperature, soil drainage, texture, depth, gravel content, CaCO<sub>3</sub> levels and slope.

**Keywords:** Soil-site suitability, ArcGIS, Ganjigatti sub-watershed, Horticultural crops

The majority of India's agriculture is rain-fed and highly erratic rains, poor soils, low yields and inadequate infrastructure development characterize it. The fragile ecologies of these rain-fed regions are likewise severely degraded. Population growth has resulted in the overexploitation of land, water and other natural resources, leading to water scarcity, soil degradation and the rapid depletion of groundwater tables (Srinivasarao et al., 2015). As a result, the agricultural growth rate is stagnant and food price inflation is soaring. In order to restore the declining trend of the qualities of different soils in India, there has been an emerging need for soil as well as land evaluation and land use planning (Ramamurthy et al., 2012, Naidu et al., 2015). The concept of soil-site suitability for crop production is central to sustainable agriculture and plays a pivotal role in ensuring global food security. Soil-site suitability analysis involves a comprehensive assessment of soil properties, environmental factors and crop requirements to determine the appropriateness of a specific location for successful crop cultivation. This multifaceted evaluation is indispensable for maximizing crop yields, minimizing resource inputs and sustaining the long-term health of agricultural ecosystems. For rationalizing land use, the soil-site suitability for different crops needs to be determined (Ramamurthy et al., 2020).

These suitability models provide guidelines to decide the policy of growing the most suitable crops depending on the suitability and capability of each soil unit. There is need to identify land-specific suitability criteria for different annual crops through a land evaluation approach with the help of soil parameters and yield data.

Remote sensing (RS) data offers the capability to identify and characterize various physiographic features, along with providing supplementary data regarding site attributes such as slope, aspect, and orientation within the study area. However, when it comes to assessing crop suitability, a more comprehensive understanding of soil profile properties becomes imperative. Therefore, soil survey data plays an indispensable role in creating a detailed soil map specific to the region under consideration. This map, in turn, serves as a crucial tool for determining the suitability of the area for different crops and for conducting an analysis of suitable cropping systems. By integrating RS data with information derived from soil surveys, it is possible to harness the power of Geographic Information Systems (GIS) to assess crop suitability across a range of soil types and biophysical conditions. This integrated approach, as demonstrated in previous studies (Hegde et al., 2019, Tuyen et al., 2019, Chikkaramappa et al., 2020), offers significant potential for

quantitative land evaluation. The research presented here focuses on showcasing the synergistic use of remote sensing (RS) and Geographic Information System (GIS) data to evaluate the suitability of sites for cultivating specific horticultural crops in the Ganjigatti sub-watershed of Karnataka. The crops examined in this study include chili, tomato, rose and jasmine.

## MATERIAL AND METHODS

**Site location:** The study was conducted in 2021–2023, in the Ganjigatti sub-watershed (5B1A4F) of Dharwad district in Karnataka, situated between 15° 10' 10.114" to 15° 17' 1.147" N latitudes and 75° 0' 57.672" to 75° 4' 50.525" E longitudes, with the highest elevation of 610 m above mean sea level. The total geographical area of the watershed is about 4323.84 ha. The annual temperature ranges from 24.68 to 26.67 °C. The average rainfall in the watershed was 917.00 mm (Fig. 1). Relative humidity varies from 28% in summer to 70% in winter. The average potential evapotranspiration (PET) is 150 mm and varies from 115 to 232 mm. The PET is always higher than precipitation in all the months except August and October. Generally, the length of crop growing period (LGP) is 150 days and starts from 3<sup>rd</sup> week of June to third week of November. After preliminary traversing of the entire watershed using a 1:7,920 scale base map and satellite imagery, based on geology, drainage pattern, surface features, slope characteristics, land use, landforms and physiographic divisions, twenty-seven (27) soil profiles were selected and studied and their morphometric characteristics were recorded.

**Soil properties:** Physical and chemical properties were

estimated using standard procedures. The detailed soil resource inventory of the Ganjigatti sub-watershed was carried out and 21 series mapped into sixty-one (61) mapping units based on surface soil properties. After a detailed soil survey, crop suitability maps for major fruit crops growing in the Ganjigatti sub-watershed area at soil phase level were prepared by using the platform of ArcGIS 10.8.2. Their suitability was assessed using the limitation method regarding the number and intensity of limitations (Naidu et al., 2006). This evaluation procedure consists of three phases. In phase I, the data was collected in terms of characteristics (Table 1). The landscape and soil characteristics used to evaluate soil suitability: topography (slope %), wetness (flooding and drainage), physical soil characteristics (texture/structure, % coarse fragments by volume, soil depth in cm, CaCO<sub>3</sub> per cent), salinity (EC, dSm<sup>-1</sup>) and alkalinity (ESP). The study locations were nearly level to moderately steep sloping and had never been flooded (F0). The drainage conditions were moderately well to well and sandy loam to clay in texture, as per the guidelines given by FAO (1976). Weighted mean of each property was calculated and soil-site characteristics of different soil units were obtained. These weighted average data have been used to evaluate and soil site suitability (FAO 1976). In phase II, the landscape and soil requirements for these five crops were taken from Naidu et al (2006) as described by Sehgal (2005). In phase III, the land suitability under rainfed conditions has been assessed by comparing the landscape and soil characteristics with crop requirements at different limitations levels: no (0), slight (1), moderate (2), severe (3), and very severe (4). Limitations are deviations from the optimal conditions of a land characteristic, such as land quality, that adversely affect the kind of land use. If a land characteristic is optimal for plant growth, it has no limitation. On the other hand, when the same characteristic is unfavourable for plant growth, it has severe limitations for land evaluation types. Thus, the evaluation was done by comparing the land characteristics with the limitation levels of the crop requirement given by Naidu et al. (2006), as described by Sehgal (2005). The number and degrees of limitations suggested the suitability class of each soil series for a particular crop, as given by FAO (1976).

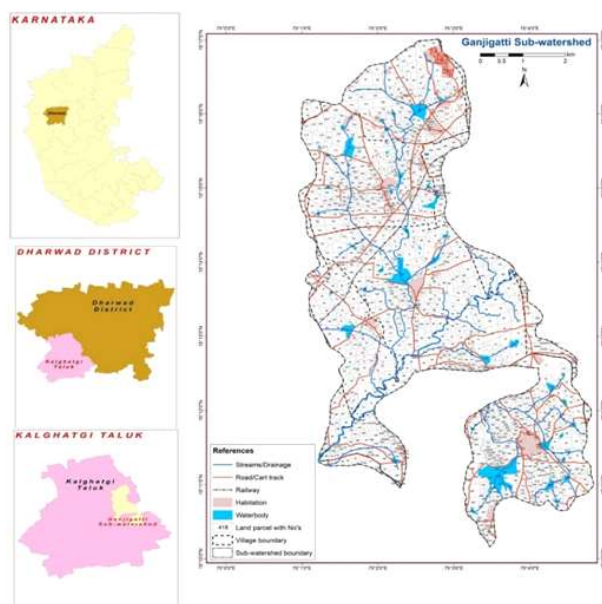


Fig. 1. Location of the study area

## RESULTS AND DISCUSSION

Soil-site suitability evaluation consider the topography (t) of the land (slope% and erosion), soil physical conditions (s) (surface texture, stoniness, gravelines, CaCO<sub>3</sub> content and depth), wetness (w) (drainage) and fertility parameters (f) (CEC, BS, OC and EC) (Sehgal 2005). Fertility limitations (f) were not considered for soil-site suitability evaluation of sub-

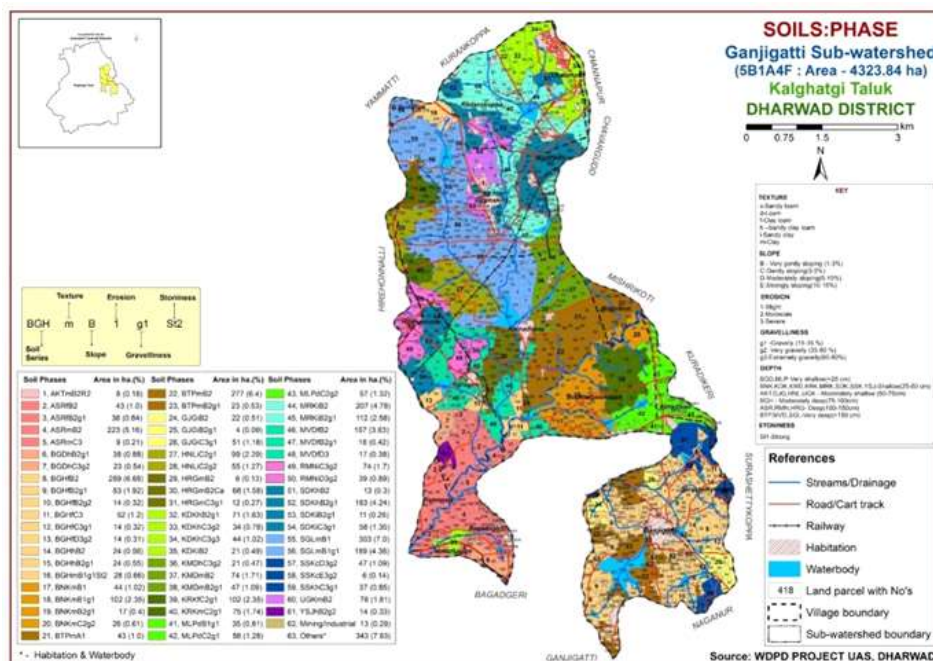
**Table 1.** Soil-site characteristics of soil mapping units of Ganjigattisub-watershed

Soil phases	Wetness (w)	Physical condition of soil (s)					Fertility (f)				Salinity/Alkalinity (n)		Erosion (e)
	Drainage	Texture	Depth (cm)	Stoniness	Gravel (%)	CaCO <sub>3</sub> (%)	pH	OC (%)	CEC	BS (%)	EC (dS m <sup>-1</sup> )	ESP (%)	Slope %
AKTmB2R2	Moderately well	Clay	79	Nil	<15	3.21	7.18	0.48	59.33	72.52	0.28	1.48	1-3
ASRfB2	Well drained	Clay loam	130	Nil	<15	15.96	8.68	0.33	48.70	87.46	0.36	1.00	1-3
ASRfB2g1	Well drained	Clay loam	130	Nil	15-35	15.96	8.68	0.33	48.70	87.46	0.36	1.00	1-3
ASRmB2	Well drained	Clay	130	Nil	<15	15.96	8.68	0.33	48.70	87.46	0.36	1.00	1-3
ASRmC3	Well drained	Clay	130	Nil	<15	15.96	8.68	0.33	48.70	87.46	0.36	1.00	3-5
BGDhB2g1	Well drained	Sandy clay loam	20	Nil	15-35	1.5	5.91	1.19	16.55	60.76	0.49	1.94	1-3
BGDhC3g2	Well drained	Sandy clay loam	20	Nil	35-60	1.5	5.91	1.19	16.55	60.76	0.49	1.94	3-5
BGHfB2	Moderately well	Clay loam	90	Nil	<15	7.35	7.33	0.6	23.64	85.7	0.3	2.19	1-3
BGHfB2g1	Moderately well	Clay loam	90	Nil	15-35	7.35	7.33	0.6	23.64	85.7	0.3	2.19	1-3
BGHfB2g2	Moderately well	Clay loam	90	Nil	35-60	7.35	7.33	0.6	23.64	85.7	0.3	2.19	1-3
BGHfC3	Moderately well	Clay loam	90	Nil	<15	7.35	7.33	0.6	23.64	85.7	0.3	2.19	3-5
BGHfC3g1	Moderately well	Clay loam	90	Nil	15-35	2.99	7.08	0.58	23.48	83.88	0.12	1.72	3-5
BGHfD3g2	Moderately well	Clay loam	90	Nil	35-60	2.99	7.08	0.58	23.48	83.88	0.12	1.72	5-10
BGHhB2	Moderately well	Sandy clay loam	90	Nil	<15	7.35	7.33	0.6	23.64	85.7	0.3	2.19	1-3
BGHhB2g1	Moderately well	Sandy clay loam	90	Nil	15-35	7.35	7.33	0.6	23.64	85.7	0.3	2.19	1-3
BGHmB1g1St2	Moderately well	Clay	80	1-3	15-35	3.5	6.83	0.83	43.84	77.7	0.18	0.53	1-3
BNKmB1	Well drained	Clay	35	Nil	<15	2.81	7.23	0.53	28.34	69.91	0.22	2.42	1-3
BNKmB1g1	Well drained	Clay	35	Nil	15-35	2.81	7.23	0.53	28.34	69.91	0.22	2.42	1-3
BNKmB2g1	Well drained	Clay	35	Nil	15-35	2.81	7.23	0.53	28.34	69.91	0.22	2.42	1-3
BNKmC2g2	Well drained	Clay	35	Nil	35-60	2.81	7.23	0.53	28.34	69.91	0.22	2.42	3-5
BTPmA1	Well drained	Clay	200	Nil	<15	12.02	8.3	0.75	58.22	90.28	0.16	2.67	0-1
BTPmB2	Well drained	Clay	200	Nil	<15	12.02	8.3	0.75	58.22	90.28	0.16	2.67	1-3
BTPmB2g1	Well drained	Clay	180	Nil	15-35	13.28	7.82	0.45	37.39	89.51	0.36	2.63	1-3
GJGiB2	Moderately well	Sandy clay	55	Nil	<15	3.76	7.22	0.66	22.68	71.53	0.22	1.48	1-3
GJGiB2g1	Moderately well	Sandy clay	55	Nil	15-35	3.76	7.22	0.66	22.68	71.53	0.22	1.48	1-3
GJGiC3g1	Moderately well	Sandy clay	55	Nil	15-35	3.76	7.22	0.66	22.68	71.53	0.22	1.48	3-5
HNLiC2g1	Moderately well	Sandy clay	67	Nil	15-35	3.06	5.92	0.66	19.82	50.72	0.26	2.03	3-5
HNLiC2g2	Moderately well	Sandy clay	67	Nil	35-60	3.06	5.92	0.66	19.82	50.72	0.26	2.03	3-5
HRGmB2	Moderately well	Clay	130	Nil	<15	15.9	8.1	0.49	49.58	90.85	0.34	0.86	1-3
HRGmB2Ca	Moderately well	Clay	130	Nil	<15	15.9	8.1	0.49	49.58	90.85	0.34	0.86	1-3
HRGmC3g1	Moderately well	Clay	130	Nil	15-35	15.9	8.1	0.49	49.58	90.85	0.34	0.86	3-5
KDKhB2g1	Moderately well	Sandy clay loam	49	Nil	15-35	2.89	6.42	0.53	59.33	72.52	0.16	0.84	1-3
KDKhC3g2	Moderately well	Sandy Clay loam	49	Nil	35-60	2.89	6.42	0.53	59.33	72.52	0.16	0.84	3-5
KDKhC3g2	Moderately well	Sandy Clay loam	49	Nil	35-60	2.89	6.42	0.53	59.33	72.52	0.16	0.84	3-5
KDKhC3g3	Moderately well	Sandy Clay loam	49	Nil	60-80	2.89	6.42	0.53	59.33	72.52	0.16	0.84	3-5
KDKiB2	Well drained	Clay loam	35	Nil	<15	2.53	6.84	0.76	23.00	69.1	0.22	1.87	1-3
KMDhC3g2	Well drained	Sandy Clay loam	35	Nil	35-60	0.91	5.36	0.73	11.15	49.67	0.1	1.82	3-5
KMDmB2	Well drained	Clay	35	Nil	<15	0.91	5.36	0.73	11.15	49.67	0.1	1.82	1-3
KDKiB2	Well drained	Clay loam	35	Nil	<15	2.53	6.84	0.76	23	69.1	0.22	1.87	1-3
KMDhC3g2	Well drained	Sandy Clay loam	35	Nil	35-60	0.91	5.36	0.73	11.15	49.67	0.1	1.82	3-5
KMDmB2	Well drained	Clay	35	Nil	<15	0.91	5.36	0.73	11.15	49.67	0.1	1.82	1-3
KMDmB2g1	Well drained	Clay	35	Nil	15-35	0.91	5.36	0.73	11.15	49.67	0.1	1.82	1-3
KRKfC2g1	Well drained	Clay loam	30	Nil	15-35	2.65	5.61	0.58	20.03	68.16	0.09	2.07	3-5

Cont...

**Table 1.** Soil-site characteristics of soil mapping units of Ganjigattisub-watershed

Soil phases	Wetness (w)		Physical condition of soil (s)					Fertility (f)			Salinity/Alkalinity (n)		Erosion (e)
	Drainage	Texture	Depth (cm)	Stoniness	Gravel (%)	CaCO <sub>3</sub> (%)	pH	OC (%)	CEC	BS (%)	EC (dS m <sup>-1</sup> )	ESP (%)	Slope %
KRkmC2g1	Well drained	Clay	40	Nil	15-35	3.33	6.02	1.02	21.16	70.31	0.06	0.79	3-5
MLPdB1g1	Moderately well	loam	20	Nil	15-35	1.35	5.83	0.58	23.62	49.72	0.11	2.22	1-3
MLPdC2g1	Moderately well	loam	20	Nil	15-35	1.35	5.83	0.58	23.62	49.72	0.11	2.22	3-5
MLPdC2g2	Moderately well	loam	20	Nil	35-60	1.35	5.83	0.58	23.62	49.72	0.11	2.22	3-5
MRKiB2	Moderately well	Sandy Clay	28	Nil	<15	3.21	7.18	0.48	26.49	64.29	0.28	1.48	1-3
MRKiB2g1	Moderately well	Sandy Clay	28	Nil	15-35	3.21	7.18	0.48	26.49	64.29	0.28	1.48	1-3
MVDfB2	Well drained	Clay loam	170	Nil	<15	4.66	6.62	0.52	23.96	84.18	0.2	2.07	1-3
MVDfB2g1	Well drained	Clay loam	170	Nil	15-35	4.66	6.62	0.52	23.96	84.18	0.2	2.07	1-3
MVDfD3	Well drained	Clay loam	170	Nil	<15	4.66	6.62	0.52	23.96	84.18	0.2	2.07	5-10
RMNiC3g2	Well drained	Sandy Clay	120	Nil	35-60	3.2	8.28	0.64	17.35	90.34	0.18	4.65	3-5
RMNiD3g2	Well drained	Sandy Clay	120	Nil	35-60	3.2	8.28	0.64	17.35	90.34	0.18	4.65	5-10
SDKhB2	Moderately well	Sandy clay loam	39	Nil	<15	3.81	6.45	0.56	27.41	69.55	0.16	0.81	1-3
SDKhB2g1	Moderately well	Sandy clay loam	39	Nil	15-35	3.81	6.45	0.56	27.41	69.55	0.16	0.81	1-3
SDKiB2g1	Moderately well	Sandy Clay	46	Nil	15-35	3.85	6.68	0.72	19.92	87.4	0.14	2.61	1-3
SDKiC3g1	Moderately well	Sandy Clay	46	Nil	15-35	3.85	6.68	0.72	19.92	87.4	0.14	2.61	3-5
SGLmB1	Moderately well	Clay	180	Nil	<15	15.05	8.13	0.45	53.97	92.99	0.21	1.36	1-3
SGLmB1g1	Moderately well	Clay	180	Nil	15-35	15.05	8.13	0.45	53.97	92.99	0.21	1.36	1-3
SSKcD3g2	Moderately well	Sandy loam	30	Nil	35-60	1.25	5.49	0.47	6.18	69.69	0.28	2.56	5-10
SSKcE3g2	Moderately well	Sandy loam	30	Nil	35-60	1.25	5.49	0.47	6.18	69.69	0.28	2.56	10-15
SSKhC3g1	Moderately well	Sandy clay loam	30	Nil	15-35	1.25	5.49	0.47	6.18	69.69	0.28	2.56	3-5
UGKmB2	Moderately well	Clay	65	Nil	<15	3.05	7.13	0.64	29.03	58.28	0.24	1.81	1-3
YSJhB2g2	Moderately well	Sandy clay loam	30	Nil	35-60	0.45	5.55	0.64	14.54	40.22	0.12	1.86	1-3



**Fig. 2.** Soil phases of series in Ganjigatti sub-watershed

watershed since they are manageable constraints. Hence, in this soil-site suitability evaluation, soil depth, extent of erosion, surface soil texture, stoniness, gravellines, CaCO<sub>3</sub> content and drainage account for more priority. Nasre et al. (2013), Naveen Kumar et al. (2018) and Yadav et al. (2023) also employed very similar parameters in the land evaluation of the respective study sites.

Slope is the basic element for analyzing and visualizing landform characteristics. The result of classified slope reveals that most of the area is gently sloping (3-5%) and very gently sloping (1-3%) in the sub-watershed (Fig. 3). Drainage is an important determining factor in plant growth. Moderate and well-drainage facilitate plant growth while poor and excess drainage inhibits plant growth. Because of this reason, drainage was also another important factor considered for evaluation of soil-site suitability in the sub-watershed. Most of the soils in the present study are well and moderately well drained. Soil depth plays a pivotal role in soil-site suitability evaluation and land use planning because it directly influences the land's suitability for various purposes, crop selection and the overall health of ecosystems. Understanding soil depth helps optimize land use while minimizing the risk of soil erosion and environmental degradation. Soil depth can be related with a number of associated factors such as low availability of soil, restricted rooting depth and inadequate nutrient uptake. The soil depth varies from very shallow (< 25 cm), shallow (25-50 cm), moderately shallow (50-75 cm), moderately deep (75-100 cm), deep (100-150 cm) and very deep (> 150 cm) (Fig. 4), accounting for 4.83, 31.6, 7.32, 14.5, 9.8 and 23.72%, respectively. Soil texture is the relative proportions of clay, silt and sand. The soil textures in the sub-watershed are sandy loam (1.23%), loam (3.41%), clay loam (19.38%), sandy clay loam (12.16%), sandy clay (17.4%) and clay (38.2%) (Fig. 5). The soil properties of the study area were compared with the soil suitability criteria for major horticultural crops grown in North Karnataka (Table 2).

#### Soil-site Suitability Evaluation

**Chilli:** Chilli requires a warm, humid climate during the early stages and dry weather towards maturity. It can be successfully grown up to 2000 m above MSL. The chilli crop requires an annual rainfall of 750–900 mm, a soil depth of more than 75 cm, sandy loam, silt loam, clay loam, loam texture, soils free of salinity and alkalinity, and well-drained soils. The most suitable temperature for germination and growth is 25 °C to 32 °C. The length of the growing period for optimum crop production is more than 150 days (Naidu et al., 2006). The suitability of soil phases in the Ganjigatti sub-watershed for growing chilli indicated that all the mapping units were moderately suitable to currently not suitable (N),

having moderate, severe and very severe limitations of climate, soil drainage, soil physical properties and land forms. Areas of moderately (S2), marginally (S3) and currently not suitable (N) classes for chilli were 2181 (50.46%), 1571 (36.33%) and 215 ha (4.97%), respectively (Fig. 7). Based on the specific type of limitations present in the mapping units, the S2 class has been subdivided into the following sub-classes: S2c, S2cs, S2cw, S2cse, S2cwe, S2cws, and S2cwse. These sub-classes cover 4.63, 14.34, 7.23, 0.21, 1.20, 18.79 and 4.06% of the total geographical area, respectively (Tables 1, 2). Based on the type of limitations, the soil site suitability class S3 (marginally suitable) was subdivided into subclasses S3e (0.38% of TGA), S3s (33.66%) and S3se (2.29%). The sub-classes Ns (BGDhB2g1, BGDhC3g2, MLPdB1g1, MLPdC2g1 and MLPdC2g2) and Ne (SSKcE3g2) are currently not suitable for chilli cultivation due to limitations in soil physical factors such as depth, gravellines and slope percent, respectively. Patil et al. (2008) observed similar findings in a sub-watershed of the northern dry zone of Karnataka.

**Tomato:** Tomato is a warm season crop and it requires an annual rainfall of 600–750 mm, a soil depth of more than 75 cm, sandy loam, silt loam, clay loam, loam texture, soils free of salinity and alkalinity and well-drained soils. The most suitable temperature for germination and growth is 25°C to 32°C. The length of the growing period for optimum crop production is more than 150 days (Naidu et al., 2006). The majority area of the sub-watershed was moderately (S2) and marginally (S3) suitable for tomato production, at 1294 (29.92%) and 2460 (56.88%), respectively, with moderate to severe limitations in terms of length of the growing period, soil drainage, soil texture, depth, gravelines, CaCO<sub>3</sub> content and sloppiness. Only a small, significant area of 215 ha (4.97%) is currently not suitable class (N) due to the very severe limitations of soil depth and sloppiness (Fig. 8). Due to moderate limitations related to climatic factors (c), soil drainage (w), soil physical factors (s) and slope percentage (e), S2 class has been subdivided into the following sub-classes: S2c (3.63%), S2cs (8.35%), S2cws (12.95%), and S2cwse (4.99%) (Tables 1, 2). For areas with severe limitations, the soil suitability class S3 has been divided into the sub-classes: S3e (1.47%), S3s (54.21%), and S3se (1.20%). Similar constraints for tomato cultivation in the Koppal district were identified by Patil et al. (2008).

**Rose:** Rose garden requires a soil depth of more than 100 cm, sandy loam, silt loam, clay loam, loam texture, soils free of salinity and alkalinity and well-drained soils. The most suitable temperature for rose cultivation is 25 °C to 30 °C (Naidu et al., 2006). The suitability of soil phases in the Ganjigatti sub-watershed for growing rose indicated that all

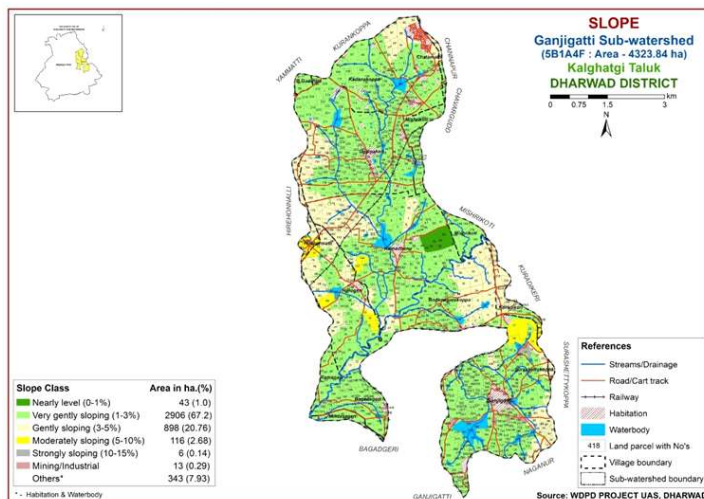


Fig. 3. Percent slope map of the Ganjigatti sub-watershed

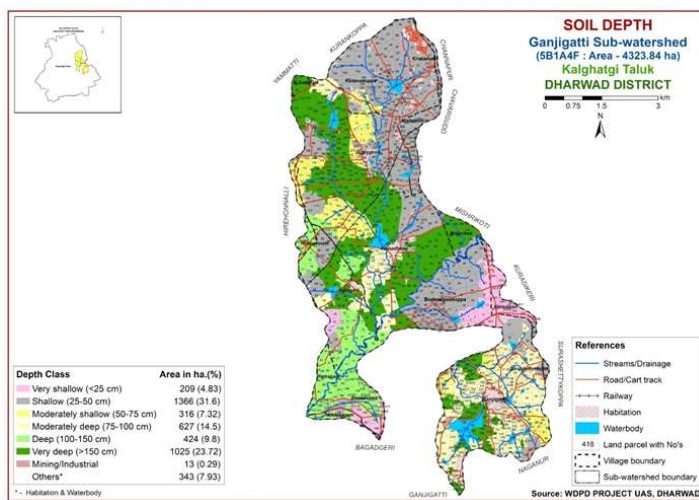


Fig. 4. Soil depth categories map of the Ganjigatti sub-watershed

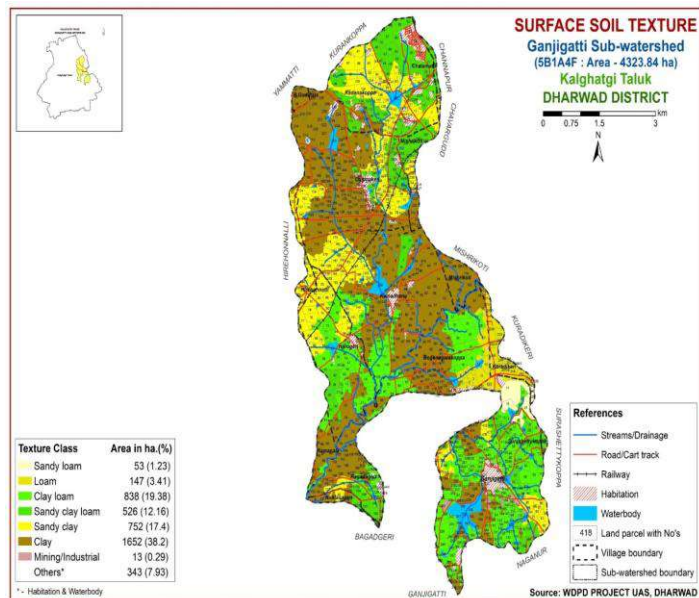


Fig. 5. Soil textural map of the Ganjigatti sub-watershed

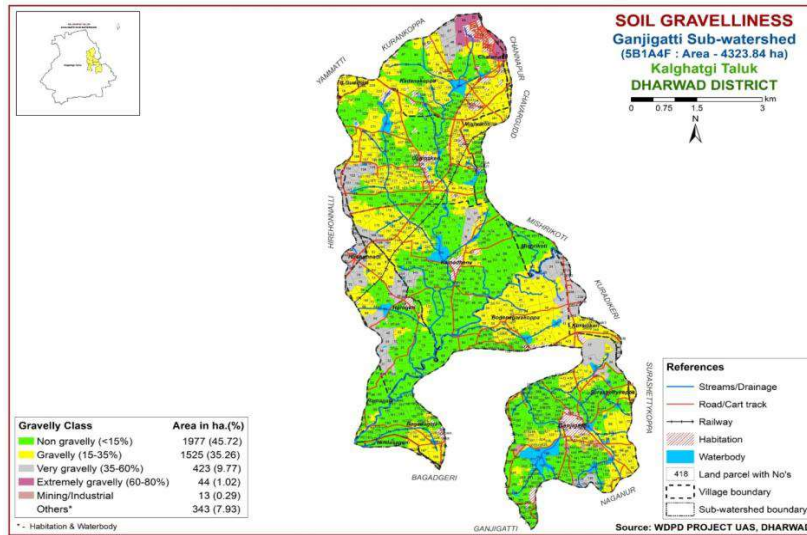


Fig. 6. Soil gravelliness map of Ganjigatti sub-watershed

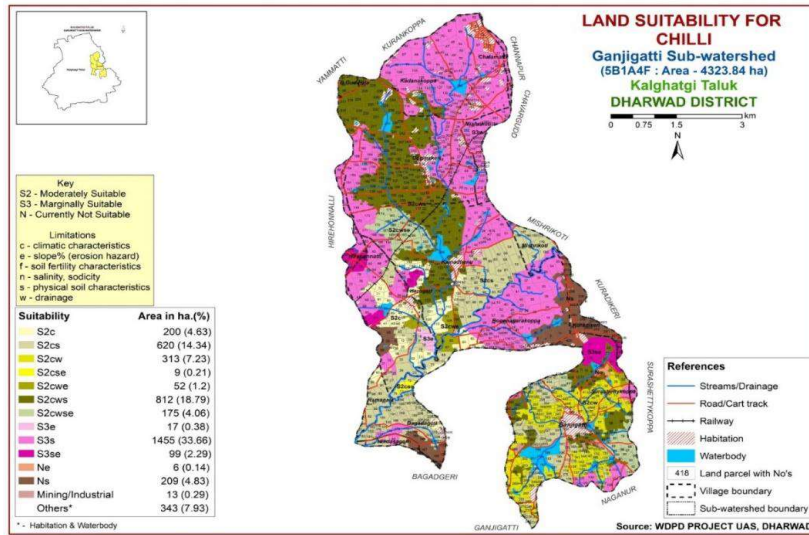


Fig. 7. Soil sitesuitability map for Chilli crop in Ganjigatti sub-watershed

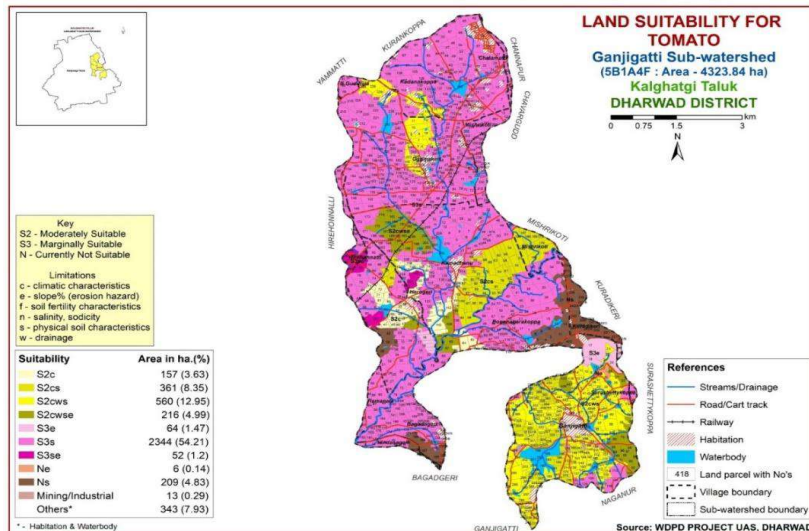


Fig. 8. Soil sitesuitability map for Tomato crop in Ganjigatti sub-watershed

**Table 2.** Soil-site suitability classification of mapping units for horticultural crops

Soil phases	Chilli	Tomato	Rose	Jasmine
AKTmB2R2	S2cws	S2cws	S2ws	S2cws
ASRfB2	S2c	S3s	S3s	S3s
ASRfB2g1	S2cs	S3s	S3s	S3s
ASRmB2	S2cs	S3s	S3s	S3s
ASRmC3	S2cse	S3s	S3s	S3s
BGDhB2g1	Ns	Ns	Ns	Ns
BGDhC3g2	Ns	Ns	Ns	Ns
BGHfB2	S2cw	S2cws	S2ws	S2cws
BGHfB2g1	S2cws	S2cws	S2ws	S2cws
BGHfB2g2	S3s	S3s	S3s	S3s
BGHfC3	S2cwe	S2cwse	S2ws	S2cws
BGHfC3g1	S2cwse	S2cwse	S2ws	S2cws
BGHfD3g2	S3se	S3se	S3s	S3s
BGHhB2	S2cw	S2cws	S2ws	S2cws
BGHhB2g1	S2cws	S2cws	S2ws	S2cws
BGHmB1g1St2	S2cws	S2cws	S2ws	S2cws
BNKmB1	S3s	S3s	Ns	S3s
BNKmB1g1	S3s	S3s	Ns	S3s
BNKmB2g1	S3s	S3s	Ns	S3s
BNKmC2g2	S3s	S3s	Ns	S3s
BTPmA1	S2cs	S2cs	S2s	S2cs
BTPmB2	S2cs	S2cs	S2s	S2cs
BTPmB2g1	S2cs	S2cs	S2s	S2cs
GJGiB2	S2cws	S2cws	S3s	S2cws
GJGiB2g1	S2cws	S2cws	S3s	S2cws
GJGiC3g1	S2cwse	S2cwse	S3s	S2cws
HNLiC2g1	S2cwse	S2cwse	S3s	S2cws
HNLiC2g2	S3s	S3s	S3s	S3s
HRGmB2	S2cws	S3s	S3s	S3s
HRGmB2Ca	S2cws	S3s	S3s	S3s
HRGmC3g1	S2cwse	S3s	S3s	S3s
KDKhB2g1	S3s	S3s	Ns	S3s
KDKhC3g2	S3s	S3s	Ns	S3s
KDKhC3g3	S3s	S3s	Ns	S3s
KDKiB2	S3s	S3s	Ns	S3s
KMDhC3g2	S3s	S3s	Ns	S3s
KMDmB2	S3s	S3s	Ns	S3s
KMDmB2g1	S3s	S3s	Ns	S3s
KRKfC2g1	S3s	S3s	Ns	S3s
KRKmC2g1	S3s	S3s	Ns	S3s
MLPdB1g1	Ns	Ns	Ns	Ns
MLPdC2g1	Ns	Ns	Ns	Ns

Cont...

**Table 2.** Soil-site suitability classification of mapping units for horticultural crops

Soil phases	Chilli	Tomato	Rose	Jasmine
MRKiB2	S3s	S3s	Ns	S3s
MRKiB2g1	S3s	S3s	Ns	S3s
MVDfB2	S2c	S2c	S1	S2c
MVDfB2g1	S2cs	S2cs	S2s	S2cs
MVDfD3	S3e	S3e	S2e	S2ce
RMNiC3g2	S3s	S3s	S3s	S3s
RMNiD3g2	S3se	S3se	S3s	S3s
SDKhB2	S3s	S3s	Ns	S3s
SDKhB2g1	S3s	S3s	Ns	S3s
SDKiB2g1	S3s	S3s	Ns	S3s
SDKiC3g1	S3s	S3s	Ns	S3s
SGLmB1	S2cws	S3s	S3s	S3s
SGLmB1g1	S2cws	S3s	S3s	S3s
SSKcD3g2	S3se	S3e	Ns	S3s
SSKcE3g2	Ne	Ne	Ns	S3se
SSKhC3g1	S3s	S3s	Ns	S3s
UGKmB2	S2cws	S2cws	S3s	S2cws
YSJhB2g2	S3s	S3s	Ns	S3s

the mapping units were highly suitable to currently not suitable (N), having none to slight, moderate, severe and very severe limitations of soil drainage, soil physical properties and limitations of land form characteristics. Areas of highly (S1), moderately (S2), marginally (S3) and currently not suitable (N) classes for rose were 157 (3.63%), 899 (20.79%), 1337 (30.92%) and 1575 ha (36.43%), respectively (Fig. 9). The soil suitability class S1 (MVDfB2) is highly suitable for rose cultivation, with either no limitations or only slight ones. Within the S2 class, sub-classes have been defined based on the types of limitations present: S2e (0.38%), S2s (8.35%) and S2ws (12.06%) (Tables 1, 2). The soil suitability class S3, which is marginally suitable for rose cultivation, has been subdivided into S3s sub-classes due to severe limitations in soil physical factors such as depth, CaCO<sub>3</sub> content, and gravel content (Tables 1 and 2). Areas classified under the sub-class Ns (36.43%) are currently unsuitable for rose cultivation due to significant limitations in soil physical factors such as depth, CaCO<sub>3</sub> content and gravel content. Similar findings of moderately to unsuitable conditions for rose cultivation were reported by Manjunatha et al. (2017) and D'Souza and Patil (2021).

**Jasmine:** Jasmine garden requires a soil depth of more than 75 cm, sandy loam, silt loam, clay loam, loam texture, soils free of salinity and alkalinity and well-drained soils. The most suitable temperature for jasmine cultivation is 18°C to 23°C



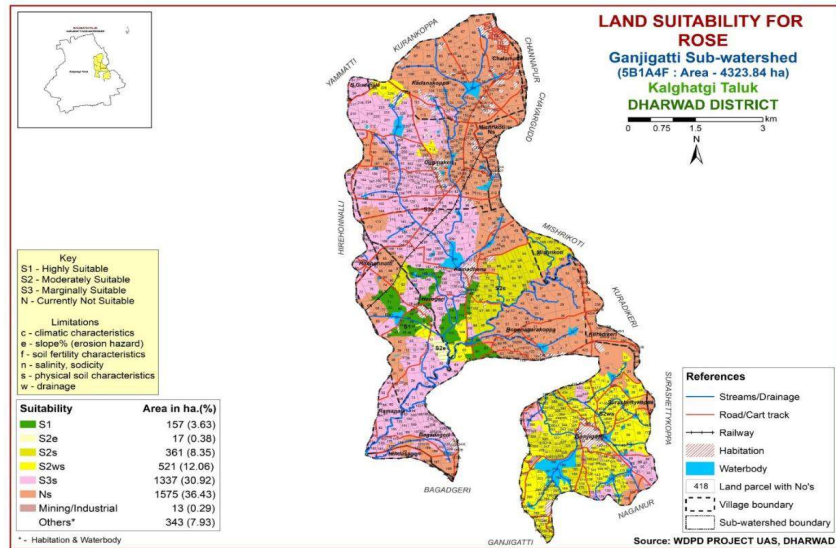


Fig. 9. Soil sitesuitability map for Rose garden in Ganjigatti sub-watershed

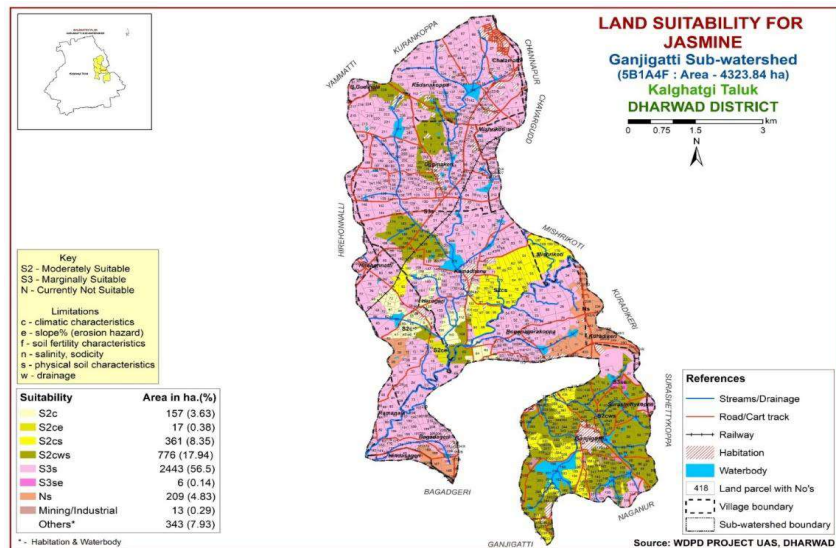


Fig. 10. Soil sitesuitability map for Jasmine garden in Ganjigatti sub-watershed

(Naidu et al., 2006). The suitability of soil phases in the Ganjigatti sub-watershed for growing jasmine indicated that all the mapping units were moderately suitable to currently not suitable (N), having moderate, severe and very severe limitations of climate, soil drainage, soil physical properties and land forms. Areas of moderately (S2), marginally (S3) and currently not suitable (N) classes for jasmine were 1311 (30.30%), 2449 (56.64%) and 209 ha (4.83%), respectively (Fig. 10). The S2 class was subdivided into S2c, S2ce, S2cs and S2cws based on the types of limitations and they covers an area of 3.63, 0.38, 8.35 and 17.94% of TGA, respectively (Table 1, 2). Based on the types of limitations, the soil site suitability class S3 was subdivided into S3s (56.50%) and S3se (0.14%). The mapping units, namely BGDhB2g1, BGDhC3g2, MLPdB1g1, MLPdC2g1 and MLPdC2g2, are

included under sub-class Ns and are currently not suitable for jasmine cultivation due to limitations in depth.

## CONCLUSION

The majority of soils in the Ganjigatti sub-watershed were found to be suitable for growing chilli, tomato, rose and jasmine, albeit to varying degrees. Most soil mapping units are moderately (S2) to marginally suitable (S3) for cultivation due to moderate to severe limitations related to climate, soil, drainage and slope. The soil series BGD and MLP are not suitable for any of the four crops due to very severe limitations in soil depth. The main limitations across all soil mapping units include shallow soil depth, slope, texture, CaCO<sub>3</sub> content and climatic factors. However, the severity of these limitations varies from slight to very severe. To identify

specific soil resource constraints for sustainable crop production in the study area, the results of this research could serve as a foundational data set. Additionally, combining remote sensing and GIS techniques could be a valuable approach for modeling crop growth and supporting sustainable land use planning decisions in the research area.

#### ACKNOWLEDGEMENT

The study is part of the WDPD project funded by Government of Karnataka. The authors duly acknowledge the financial support.

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# Enhancing Soil Quality Monitoring for Sustainable Forest Management in Northeast India: Role of Soil Quality Index in Hongmong Conservation Area, Nagaland

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**Abstract:** Forest degradation in Northeast India has raised concerns regarding soil health, particularly in regions like Nagaland, where forests are vital for indigenous communities' sustenance. This study aimed to assess soil quality and create a Soil Quality Index (SQI) in Hongmong conservation area, Mon District, Nagaland. Soil samples were collected monthly from November 2020 to October 2021 at different altitudinal zones and analyzed for various parameters. Principal Component Analysis (PCA) was used to identify a Minimum Data Set (MDS), and SQI was derived using Additive and Weighted methods. There were significant seasonal variation in soil properties across different depths and sites. Soil pH, moisture content, organic carbon, and nutrient levels were significantly influenced by altitude and seasonal changes. Soil bulk density increased with decreasing organic carbon, while clay and silt content increased with altitude. PCA identified key soil factors contributing to soil quality across sites. The creation of SQI revealed variations in soil quality, with both methods providing accurate assessments. The study underscores the importance of SQI in monitoring soil health, aiding sustainable land management practices. Findings highlight the efficacy of SQI in evaluating soil conditions and guiding conservation efforts in Nagaland's forest ecosystems.

**Keywords:** Soil quality assessment, Forest degradation, Northeast India, Soil quality index

Forest degradation in the Northeast region of India, attributed to factors such as logging, human settlement, and suboptimal land use practices, has led to a notable decline in forest cover over time (Semy et al., 2021, Temjen et al., 2022). This decline raises significant concerns, particularly considering the pivotal role forests play in sustaining the livelihoods of indigenous communities in the region (Banerjee and Madhurima 2013). Nagaland, situated in Northeast India, boasts a reported forest cover of 75.31%, upon which tribal populations heavily depend for sustenance, encompassing food, medicinal resources, and essential commodities, despite their primary involvement in Jhum cultivation (Jamir et al., 2008). The practice of Jhumming, in particular has contributed to the deterioration of forest cover in the region (Temjen et al., 2021).

Within forest ecosystems, soil is crucial in facilitating biomass production and carbon sequestration (Moffat 2003). Organic matter from forest litterfall significantly enriches soil carbon and nitrogen content (Temjen et al., 2021). However, forest soil quality remains vulnerable to adverse impacts from natural phenomena and human activities, consequently compromising its overall health (Verma and Jayakumar 2012). Therefore, an urgent need exists to establish precise soil quality indicators capable of discerning the effects of various disturbances on soil properties (Moffat 2003).

One practical approach for assessing soil quality involves the development of a Minimum Data Set (MDS) for soil

monitoring. This reduces both the cost and labor intensity associated with monitoring sites. This can be achieved by applying dimension reduction techniques such as Principal Component Analysis (PCA) (Semy et al., 2021, Temjen et al., 2022). Creating an MDS also facilitates the derivation of a Soil Quality Index (SQI), which integrates diverse soil attributes into a numerical value, aiding local stakeholders and researchers in promptly evaluating soil conditions (Mukhopadhyay et al., 2016). The SQI value ranges from zero to one, with higher values denoting superior soil quality (Mukherjee and Lal 2014). Consequently, SQI serves as a valuable tool for advancing sustainable management objectives and facilitating site productivity monitoring (Andrews et al., 2002).

Despite previous applications of SQI to assess soil quality in contexts such as Jhum lands and coal mining areas (Semy et al., 2021, Temjen et al., 2022), a notable research gap exists concerning the feasibility of employing this technique in mountainous regions, particularly those within the Mon district. Therefore, with a focus on the beneficial aspects of SQI, the present study endeavors to address this gap and contribute to the body of knowledge in this field.

## MATERIAL AND METHODS

**Study site:** The study site, Hongmong conservation area covering 3200 hectares is located at Angphang Village under Mon district, Nagaland. It is 71 km away from Mon

Headquarters. The study area is located in Mon district of Nagaland, bordered by the Tuensang district in the south, Longleng in the west, Sibsagar district of Assam in the northwest, Myanmar in the east and Longding district of Arunachal Pradesh in the northeast. This study was executed by marking three plots at different altitudinal zones. The lower zone (Site I) is at 1478 m.a.s.l N 26° 30.057'E 095° 00.317', the middle zone (Site II) at 1790m.a.s.l N 26° 29.919'E 095° 00.660' and the upper zone Site III at 2118 m.a.s.l N 26° 29.781'E 095° 00.886'.

**Soil sample collections:** From November 2020 to October 2021, the first week of each month, soil samples were taken from the three Sites. Eventually, four seasonal mean values were identified from the monthly data: Winter (November, December, and January), spring (February, March, and April), summer (May, June, and July), and autumn (August, September, and October). Samples of soil were taken at three different depths: 0–10 cm, 10–20 cm, and 20–30 cm. Air-dried soil samples were used to examine all the parameters except bulk density and soil moisture. A digital pH meter (1:5 w/v) was used to measure the pH. Table 1 depicts the various soil parameters studied.

**Selection of minimum data set:** To identify the minimum data set (MDS), conducted principal component analysis (PCA) using SPSS version 26.0. Factors resulting from varimax rotation with eigenvalues greater than 1, explaining a minimum of 5% of the data set's variation, were selected as the MDS for each site (Mandal et al., 2008). Pearson's correlation test was employed to mitigate redundancy among highly weighted variables (Yu et al., 2018). Subsequently, the MDS with the highest scores were retained from each Principal Component as per Semy et al. (2021) and Temjen et al. (2022).

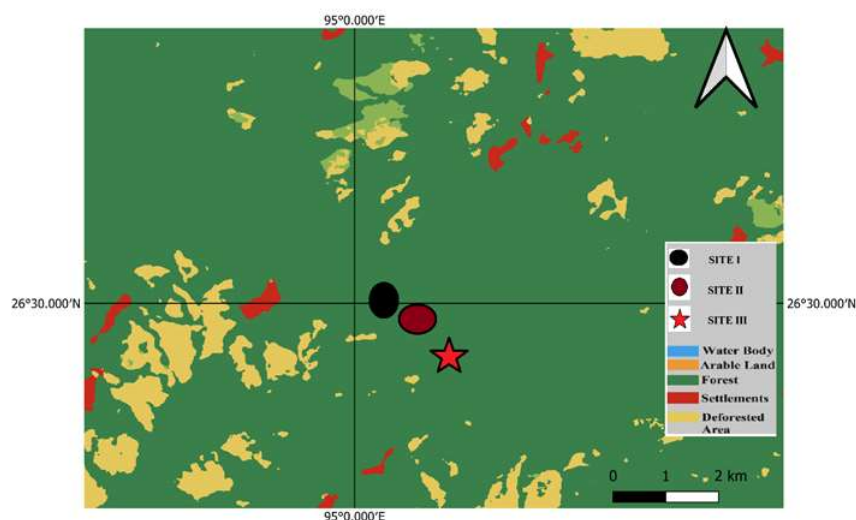
**Creation of SQI:** First, the score of each soil indicator retained in the MDS was scored as per methods utilized by Semy et al., (2021) and Temjen et al. (2022) with values ranging from zero to one via a linear scoring method. Next, the Additive quality index (Nabiollahi et al., 2017) and the weighted quality index (Raiesi 2017) were estimated to know which method provided accurate results.

**Statistical analyses:** All statistical analysis were performed using SPSS version 26.0.

## RESULTS AND DISCUSSION

### Seasonal and depth variation soil properties of each site:

For Site I, notable differences between seasons, particularly with lower pH values during winter and higher values during autumn were reported (Table 2 and 3). The significant variation was also observed in pH levels at all depths (Table 4), with soil moisture content exhibited significant variation across all depths with the highest levels recorded in autumn and the lowest in winter. Similarly, SOC content showed significant variation at all depths being highest during summer and lowest during winter.  $N_{av}$  values varied significantly at all depths with diverse seasonal trends observed. Significant variation was also noted in  $K_{ex}$  levels among different soil depths with seasonal differences apparent. Similarly, for Site II, significant variation was observed in pH levels at all depths with notable differences between seasons. Soil moisture content exhibited significant variation across all depths with distinct seasonal patterns. SOC content showed significant variation at all depths with varying levels across seasons.  $N_{av}$  values showed significant variation at specific depths, with seasonal trends evident. Significant variation was observed in  $K_{ex}$  levels at specific



**Fig. 1.** Land use map of study area displaying the three study sites

**Table 1.** Soil parameters and its protocol

Method	Parameter measured	Reference
Gravimetric method	Soil moisture	Misra 1968
Pipette method	Clay content	Piper (1942)
Core method	Bulk Density (BD)	Allen (1989)
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> wet oxidation method	Soil Organic Carbon (SOC)	Walkley and Armstrong Black (1934)
KMnO <sub>4</sub> oxidation method	Available Nitrogen (N <sub>av</sub> )	Kjeldahl (1883) / Kelplus Nitrogen Estimation System
Bray and kurtz method	Available Phosphorus (P <sub>av</sub> )	Bray's No. 1 Extract Method (Bray and Kurtz1945)
Photometric method	Exchangeable Potassium (K <sub>ex</sub> )	Trivedy and Goel (1986)

**Table 2.** Seasonal variation of soil parameters from the different sites

Site	Season	Soil depth (in cm)	pH	Moisture (%)	SOC (%)	N <sub>av</sub> (kg ha <sup>-1</sup> )	K <sub>ex</sub> (kg ha <sup>-1</sup> )	P <sub>av</sub> (kg ha <sup>-1</sup> )	BD (g cm <sup>-3</sup> )	Clay (%)	Silt (%)	Sand (%)	Porosity	Particle density
I	Winter	0-10	3.4	36.6	1.81	1434.74	214.77	22.13	1.01	18.56	7.16	74.26	21.80	1.28
		10-20	3.7	34.4	1.45	722.12	64.21	13.76	1.09	16.76	6.73	76.50	29.16	1.52
		20-30	3.7	34.5	0.93	469.63	39.55	11.69	1.20	14.20	9.83	75.96	27.23	1.62
	Spring	0-10	4.7	45.7	2.56	638.04	419.03	19.95	0.87	16.78	9.30	73.91	43.43	1.51
		10-20	4.8	39.2	2.00	525.59	216.72	15.20	1.02	11.07	6.67	82.25	36.06	1.57
		20-30	4.9	37.3	1.73	394.16	201.60	18.49	1.07	10.15	7.69	82.15	27.93	1.50
	Summer	0-10	4.2	45.7	2.93	864.46	306.57	22.23	0.98	17.66	7.83	74.50	38.73	1.62
		10-20	4.4	45.3	2.86	700.54	141.06	14.19	1.06	13.83	5.47	80.70	31.46	1.56
		20-30	4.6	42.2	2.26	359.17	71.99	13.05	1.22	10.47	4.53	84.99	24.36	1.64
	Autumn	0-10	4.8	47.3	2.74	950.98	455.22	29.19	1.08	21.09	10.86	68.04	38.90	1.77
		10-20	5.1	41.1	2.58	875.58	444.17	22.25	1.23	20.93	12.40	66.65	23.23	1.67
		20-30	5.3	36.3	2.37	666.71	293.41	20.31	1.29	14.47	10.89	74.63	17.00	1.56
II	Winter	0-10	3.5	33.4	1.23	1557.33	304.33	20.48	0.80	16.66	10.90	72.36	45.66	1.44
		10-20	3.7	32.9	0.98	783.61	223.24	15.28	0.94	9.46	7.53	83.00	46.20	1.68
		20-30	3.7	28.7	0.66	990.98	198.45	12.70	1.03	10.56	1.93	87.50	43.73	1.80
	Spring	0-10	4.5	44.1	2.00	1297.61	328.10	25.67	0.76	16.59	9.47	73.94	53.06	1.59
		10-20	4.6	38.3	1.6	975.01	194.33	20.94	0.94	9.73	7.56	82.69	37.16	1.49
		20-30	4.6	36.6	1.14	816.27	108.45	13.82	1.02	9.06	5.93	85.00	31.93	1.50
	Summer	0-10	4.4	55.5	2.26	1471.26	381.88	31.62	0.73	14.43	7.56	78.00	63.00	1.97
		10-20	4.4	43.1	2.13	1458.72	221.07	27.01	0.92	14.30	8.25	77.44	50.20	1.86
		20-30	4.3	41.1	2.09	824.85	196.90	21.42	1.17	10.22	3.01	86.77	38.60	1.90
	Autumn	0-10	5.1	46.2	2.33	1008.32	388.07	26.35	0.90	17.31	11.58	71.10	41.43	1.53
		10-20	5.2	40.5	2.27	925.07	250.06	20.21	1.07	12.58	6.25	81.16	23.50	1.58
		20-30	5.3	36.5	2.09	781.04	200.61	16.99	1.16	8.34	3.30	88.34	23.96	1.53
III	Winter	0-10	3.5	31.6	0.92	1632.12	340.14	20.04	6.40	18.41	9.53	72.05	52.13	1.22
		10-20	3.6	30.2	0.83	1105.40	222.61	14.49	0.65	13.07	7.70	79.22	50.03	1.27
		20-30	3.6	28.2	0.80	895.98	169.72	11.66	0.72	10.43	5.66	83.90	55.86	1.69
	Spring	0-10	4.2	42.9	1.06	1325.98	341.34	31.58	0.62	19.17	12.20	68.76	50.76	1.33
		10-20	3.9	39.2	1.03	979.71	166.28	21.18	0.74	12.20	8.26	79.52	46.63	1.39
		20-30	3.8	39.7	1.04	518.90	113.83	14.92	0.89	9.58	2.96	87.45	37.10	1.42
	Summer	0-10	4.3	52.7	1.80	1886.82	440.89	46.78	0.66	20.44	10.54	69.01	66.60	1.98
		10-20	4.5	51.6	1.70	1221.63	216.33	19.22	0.81	14.65	10.92	74.42	56.83	1.88
		20-30	4.6	49.9	1.55	795.57	154.46	16.64	0.98	13.00	8.03	78.96	47.63	1.86
	Autumn	0-10	4.6	45.6	2.11	1168.47	319.49	29.43	0.74	17.70	11.61	70.68	60.76	1.89
		10-20	4.8	38.6	2.02	987.00	200.30	20.01	0.81	12.86	7.39	79.74	61.03	1.81
		20-30	4.9	34.2	1.95	692.33	160.62	17.15	0.96	10.34	3.85	85.80	41.63	1.65

depths with distinct seasonal differences. For Site III, significant variation was observed in pH levels at all depths with seasonal differences. Soil moisture content exhibited

**Table 3.** The p-values of the seasonal variation of soil parameters from the different sites

Parameters	Soil depth (cm)	Site I	Site II	Site III
		P-value	P-value	P-value
Particle density	0-10	<b>&lt;0.001</b>	<b>.002</b>	<b>&lt;0.001</b>
	10-20	.222	<b>.001</b>	<b>&lt;0.001</b>
	20-30	.544	<b>.002</b>	.007
Porosity	0-10	.150	.095	.139
	10-20	.618	.500	.246
	20-30	.793	.117	.229
Moisture	0-10	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	10-20	<b>.001</b>	<b>.002</b>	<b>&lt;0.001</b>
	20-30	<b>.037</b>	<b>.010</b>	<b>&lt;0.001</b>
P <sub>av</sub>	0-10	.425	.610	.455
	10-20	.365	.528	.527
	20-30	.368	.532	.364
K <sub>ex</sub>	0-10	<b>&lt;0.001</b>	<b>.002</b>	.006
	10-20	<b>&lt;0.001</b>	.402	.246
	20-30	<b>&lt;0.001</b>	<b>.001</b>	.278
N <sub>av</sub>	0-10	<b>&lt;0.001</b>	<b>&lt;.001</b>	<b>.001</b>
	10-20	<b>&lt;0.001</b>	.357	.277
	20-30	<b>&lt;0.001</b>	<b>.004</b>	<b>.048</b>
BD	0-10	.425	.610	.530
	10-20	.365	.528	.527
	20-30	.368	.532	.364
SOC	0-10	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>.004</b>
	10-20	<b>.015</b>	<b>.001</b>	<b>.003</b>
	20-30	<b>.005</b>	<b>.005</b>	<b>&lt;0.001</b>
Clay	0-10	.193	.581	.164
	10-20	.002	.101	.742
	20-30	.006	.268	.245
pH	0-10	<b>&lt;0.001</b>	<b>.000</b>	<b>&lt;0.001</b>
	10-20	<b>&lt;0.001</b>	<b>.000</b>	<b>&lt;0.001</b>
	20-30	<b>&lt;0.001</b>	<b>.000</b>	<b>&lt;0.001</b>
Silt	0-10	.428	.260	.751
	10-20	.099	.881	.260
	20-30	.040	.206	.202
Sand	0-10	.274	.365	.587
	10-20	.011	.558	.363
	20-30	.016	.418	.202

Bold font indicates a significant result ( $p < 0.05$ ). Soil moisture (Moisture), available phosphorus (P<sub>av</sub>), exchangeable potassium (K<sub>ex</sub>), available nitrogen (N<sub>av</sub>), bulk density (BD), soil organic carbon (SOC), soil clay content (Clay) and soil pH (pH)

significant variation across all depths with varying levels across seasons. Soil Organic Carbon content showed significant variation at all depths, with distinct seasonal patterns. N<sub>av</sub> values varied significantly, with the highest values reported in summer and lowest in autumn, while no statistical difference was observed in K<sub>ex</sub> levels. Seasonal variation in P<sub>av</sub> levels was observed with diverse trends across seasons. Additionally, significant variation was observed in clay, silt, and sand content, with distinct seasonal patterns. Particle density and porosity of the soil exhibited significant variation across seasons and depths, highlighting diverse soil characteristics.

Seasonal variations in soil properties, such as pH, moisture content, and organic carbon, exhibit distinct patterns with higher values observed during warmer seasons due to increased moisture and decomposition activity, while colder seasons show lower values attributed to decreased microbial activity and moisture levels (Table 2). These fluctuations highlight the importance of monitoring soil parameters across seasons to understand their dynamic influence on soil health and nutrient availability (Temjen et al., 2022). Soil pH was highest in site I across all soil depths. The higher soil pH value was at the lower altitude and it decreased with the increase in altitude. This may be due to the organic matter accumulation on the top soil with the increase at higher elevation (Badia et al., 2016). Ramesh et al. (2019) and Kamal et al. (2023) also observed similar trend. The soil moisture did not show any statistical difference among the three sites, but it was slightly higher at Site III, the higher altitude. Griffiths et al. (2009), observed an increase in soil moisture with increasing elevation in the Oregon Cascade Mountains, aligning with the present study. This increased moisture at higher elevation may be due to the slow decomposition of litter on the forest. soil organic carbon (SOC) decreased with increase in elevation, this may also be attributed to the low accumulation of the organic matter at higher altitude which has lower temperature (Sheikh et al., 2009, Amir et al., 2019) observed lower soil organic carbon in higher elevations in forest soil in Garhwal Himalaya. Similar trend was observed by Bangroo et al., (2017) with altitudinal zones ranging from 1800-2500 masl at the Mawer Range Forest, Kupwara District of Jammu & Kashmir. The N<sub>av</sub>, P<sub>av</sub> and K<sub>ex</sub> showed an increased value with the altitude which may be attributed to the slow process of mineralization and decomposition at the higher elevation (Shedayi et al., 2016). The lower zone, site I has a high BD (1.19 g cm<sup>3</sup>) while the upper zone site III has low BD value (0.65 g cm<sup>3</sup>). The human activities at the base of the forest may have caused compaction of soil that has led to low Bulk density. With the decrease in organic carbon at different layers of soil, there

was an increase in the soil bulk density at all three sites. The clay and silt particle increased with increase in altitude, Site I < Site II < Site III. The soil samples contain sand, silt, and clay in the following ranges: 70.12-86.90% for sand, 3.54-10.97% for silt, and 9.55-18.93% for clay. These values fall within the ranges reported by Pravin et al. (2013). The soil porosity values were similar Yafei et al. (2022). The increasing trend of the bulk density at Site I is reflected in the porosity of the soil that decreases.

**Creation of SQI from MDS:** The result of the PCA depicts that PCA corresponding Site I, II and III explained 85.96,

80.45 and 82.36% respectively of the total data variance (Table 5). In Site I: Sand, SOC, BD and  $N_{av}$  were retained as MDS. Under Site II: Sand, SOC,  $N_{av}$  and porosity, and under Site III: Clay, pH and porosity were retained, respectively. The retention of these MDS at the particular site reflects the most influencing soil factors (Longchar et al., 2023). Workers have similarly isolated such MDS for aiding in soil monitoring activities. Temjen et al., (2022) isolated and created MDS for fallow and Jhum lands under the region and isolated similar soil traits such as SOC and pH from a total soil data set, highlighting the significant role of these soil variables in

**Table 4.** Depth-wise comparison soil parameters

Soil depth	Sites	pH	Porosity	SOC (%)	Nav (kg ha <sup>-1</sup> )	Kex (kg ha <sup>-1</sup> )	Pav (kg ha <sup>-1</sup> )	Moisture (%)	BD (g cm <sup>-3</sup> )	Clay (%)	Silt (%)	Sand (%)	Particle density (gm cm <sup>-3</sup> )
0-10 cm	Site I	4.31a	35.72a	2.51c	972.05a	348.89a	23.37a	46.39a	0.98c	16.25a	8.79a	72.68ab	1.54a
	Site II	4.38a	51.02b	1.90b	1333.63b	350.59a	26.03a	44.83a	0.800b	18.52b	9.87a	73.85b	1.63a
	Site III	4.15a	57.56b	1.45a	1710.60b	360.46a	31.96b	43.21a	0.6575a	18.93b	10.97a	70.12a	1.60a
10-20 cm	Site I	4.51a	29.98a	2.22b	705.95a	222.17a	16.35a	40.02a	1.1025c	15.65b	8.23a	76.52a	1.58a
	Site II	4.44a	50.79a	1.79ab	1073.43a	201.38a	20.86b	38.69a	.969b	11.52a	7.40a	81.07a	1.65a
	Site III	4.18a	53.63a	1.42a	1503.35a	216.53a	18.73ab	39.90a	.7558a	13.20ab	8.57a	78.22a	1.59a
20-30 cm	Site I	4.62a	24.13a	1.82a	472.41a	176.10a	16.23a	37.58a	1.19b	12.32a	7.82b	79.43a	1.58a
	Site II	4.49a	34.55b	1.49a	853.28c	149.65a	15.09a	35.71a	1.09b	9.55b	3.54a	86.90a	1.68a
	Site III	4.25a	45.55c	1.34a	725.69b	151.63a	15.89a	38.02a	.890a	10.83ab	5.13ab	84.03a	1.65a

Values in the same column with different superscripts in their respective soil depth (0-10 cm, 10-20 cm, and 2030 cm) are significantly different at 5% level by Duncan's multiple range test ( $p < 0.05$ )

**Table 5.** Principal Component Analysis (PCA) with factor loadings of the different soil parameters

Site	SITE I				SITE II				SITE III		
	PC-1	PC-2	PC-3	PC-4	PC-1	PC-2	PC-3	PC-4	PC-1	PC-2	PC-3
Eigen value	4.62	2.455	2.038	1.198	5.17	2.27	1.14	1.064	6.20	2.56	1.11
% Variance	38.54	20.45	16.79	9.82	43.10	18.92	9.56	8.86	51.67	21.39	9.29
% Cumulative frequency	38.54	59.00	75.98	85.96	43.10	62.02	71.59	80.45	51.67	73.06	82.36
Factor loadings											
$K_{ex}$	.574	.710			.768	.438			.853		.329
$P_{av}$	.566	.663			.548	.703			.746	.353	
BD			-.925		-.694			-.446	-.338	.365	-.821
Particle density			.332	.727	-.657		.391				.885
SOC		.905				.913			.664	.589	
$N_{av}$	.495			-.767			.927		.849		.346
Moisture		.777	.401		.359	.814			.419	.746	
pH		.511		.675	-.337	.766				.890	
Porosity								.897		.307	.884
Clay	.839				.862				.955		
Silt	.875				.817				.765		
Sand	-.972				-.904				-.938		

Bold indicates the highest loaded factors in their respective columns which are retained for minimum data set (MDS). PC-1 (Principal Component one), PC-2 (Principal Component two) and PC-3 (Principal Component three). soil moisture (Moisture), available phosphorus ( $P_{av}$ ), exchangeable potassium ( $K_{ex}$ ), available nitrogen (Nav), bulk density (BD), soil organic carbon (SOC), soil clay content (Clay) and soil pH (pH)

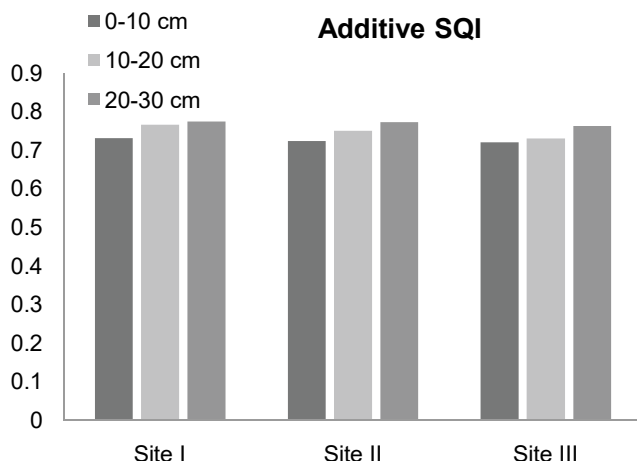


Fig. 2. Additive SQI of the three sites

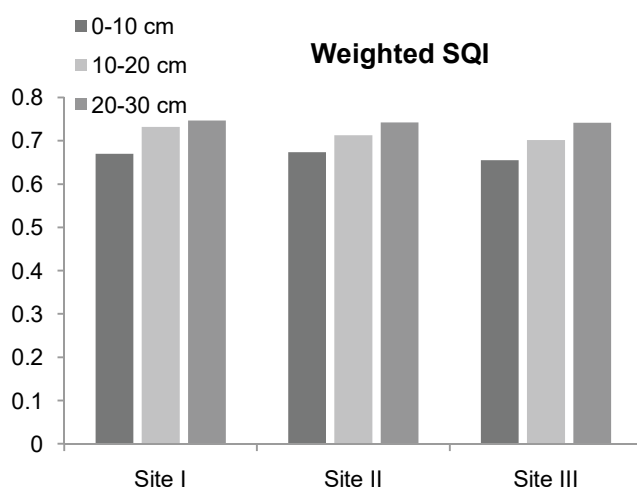


Fig. 3. Weighted SQI of the three sites

determining soil quality. Therefore, the present MDS highlight the distinct soil variable in the region.

The creation of the SQI from the MDS is depicted in Figure 2 and 3. The result of the additive SQI depict soil quality in the order III>II>I and the weighted SQI also depict in the order III>II>I. Mishra et al. (2017), Semy et al. (2021) and Temjen et al. (2022) have used the weighted index owing to its greater accuracy. But in the present study both additive and weighted method give accurate result. The utilization of SQI is effective in reducing both cost and resources associated with monitoring programs (Mamehpour et al., 2021)

### CONCLUSION

The study emphasizes the importance of assessing soil quality in forest ecosystems, particularly in Northeast India where forest degradation threatens biodiversity and community livelihoods. Through techniques like Principal

Component Analysis and the development of a Minimum Data Set (MDS), have shown a practical approach to monitoring soil health, crucial for informing sustainable land management. There was distinct seasonal variations in soil properties across different altitudinal zones, highlighting the dynamic nature of soil processes and the need for continuous monitoring. Creating a Soil Quality Index (SQI) from the MDS provides a comprehensive tool for evaluating soil conditions promptly, aiding in effective soil conservation and management. This study fills a research gap by demonstrating the feasibility of SQI techniques in mountainous regions like the Mon district of Nagaland. By enhancing understanding of soil dynamics in challenging terrains, can equip stakeholders and researchers with valuable insights for implementing evidence-based soil conservation strategies. Ultimately, preserving soil quality in forest ecosystems is essential for sustaining biodiversity, ecosystem services, and the livelihoods of indigenous communities. Continued efforts in monitoring soil health and implementing evidence-based management practices are crucial for promoting resilience and sustainability amidst ongoing environmental challenges.

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# Morphophysiological Responses of Rice (*Oryza sativa* L.) Genotypes to Salinity Stress during Germination Stage

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**Abstract:** Salinity hinders seed germination and causes a delay in emergence, diminishes photosynthesis, promotes senescence, and eventually reduces crop yields. This study examined the impact of soil salinity levels (0, 100, and 200 mM) on seed germination and seedling growth in seven rice cultivars. The parameters, viz. germination rate, mean germination time, root and shoot length, root and shoot fresh weight, and seedling height stress index, assessed during the germination and early seedling phases were found to be influenced by salinity, genotypes, and their interactions. Results showed that increased salinity levels led to an increase in germination time and a decrease in germination index. According to the study's findings, cultivars MR211, MR220, and Pokkali had the highest values for the characteristics being examined. In contrast, all cultivars showed a decline in these characteristics as saline levels rose. Under conditions of salt stress, genotypes MR211, MR220, and Pokkali outperformed other genotypes in terms of seedling growth in root and shoot length. Based on their germination index and average germination time, Pokkali and BR29 can be categorised as salt-tolerant and salt-sensitive, respectively.

**Keywords:** Seed germination, Salinity, Seedling growth, Morphophysiological response

Several environmental factors act as inhibitors of plant growth, with salt emerging as a prominent limitation (Ashraf 2004, Munns et al., 2006). Soil salinity is one of the most detrimental effects of climate change on coastal agricultural land, as rising sea levels have increased salinity from 1 to 33% over the last 25 years (Rahman et al., 2018). Plants exhibit heightened vulnerability to environmental stresses during seed germination and early seedling growth (Ashrafuzzaman et al., 2003). A negative correlation between seed germination and increasing salinity levels has been well demonstrated in previous studies. This decline in germination can occur through two mechanisms: osmotically, by reducing water absorption, or ionically, by promoting the accumulation of sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ions. Consequently, this imbalance in nutrient uptake and the presence of toxic ions can hinder the germination process (Sarwar et al., 2020, Tobe et al., 2002). Salinity inhibits the process of seed germination, retards the plant growth, and diminishes the overall yield of crops.

Cereal crops e.g., wheat, maize, rice, and barley, are classified as glycophytes (salt-sensitive), however, salt tolerance can differ both between species and within a species (Shelden and Munns 2023). Rice (*Oryza sativa* L.) holds significant global importance, serving as a dietary staple for more than half of the world's population. There is considerable interest in developing rice cultivars that are both high-yielding and resistant to salinity. Pokkali and Nonabokra are two rice cultivars known for their salt tolerance; nonetheless,

production potential is somewhat lower when compared to contemporary rice types. Malaysian Agricultural Research and Development Institute, like other rice-growing countries, has released several high-yielding rice cultivars, including MR84, MR211, MR219, MR220, and MR232. However, the salinity tolerance of these rice cultivars has not yet been verified.

Many studies have been conducted to elucidate rice plants' physiological responses to NaCl salinity stress (Liu et al., 2018). Rice cultivars are salt tolerant during germination, though salinity retards germination, but crop yield is affected due to its increased sensitivity to salinity during early seedling growth. Significant variation in seed germination occurs between rice cultivars grown in salinity conditions, (Liu et al., 2018) extensively. The objective of this study was to investigate the physiology of salt tolerance in rice plants, to assess the tolerance level of Malaysian high-yielding rice cultivars to salinity during germination and early seedling stage in the laboratory before field trials, and to identify cultivars with a high potential for breeding salt-tolerant lines.

## MATERIAL AND METHODS

Laboratory experiment was conducted to study germination and seedling growth of different rice cultivars under different salinity levels. Seven rice cultivars namely MR84, MR211, MR219, MR220, MR232, Pokkali, and BR29, were used in this study. The seeds were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min and rinsed several times with distilled water. The surface

sterilized seeds were placed on Whatman no.1 filter paper lined in the Petri dishes. Twenty-five seeds were kept at equidistance in each Petri dish (9 cm diameter). The filter papers were moistened with saline solutions of 100 and 200 mM. Distilled water-moistened Petri dishes served as control. Petri dishes were sealed with Parafilm and placed on the laboratory bench at room temperature 24-28°C with a 12-hour photoperiod for germination up to ten days. Each treatment was replicated three times in a completely randomized experimental design.

Germination was observed daily according to the recommendation of the International Seed Testing Association (ISTA 1993). Seeds exhibiting radicle emergence (>2 mm) were recorded every day until germination ceased. The total number of seed germinated were counted and the percentage was calculated. The germination index (GI) was calculated after final germination with the following equation:

$$GI = \frac{\text{Germination \% in each treatment}}{\text{Germination \% in the control}} \times 100$$

The mean germination time (MGT) was calculated according to the equation proposed by Ellis and Roberts (1981), as follows:

$$MGT = \frac{\sum Dn}{\sum n}$$

Where MGT is the mean germination time, n is the number of seeds, which were germinated on day D; D is the number of days counted from the beginning of germination.

The rate of germination was estimated using a modified Timson index of germination velocity =  $\sum G/t$ , where G is the percentage of seed germination at one-day intervals and t is the total germination period (Khan and Ungar 1984). The maximum possible value using this index with our data was 50 (i.e. 1000/20). The higher the value, the more rapid the rate of germination.

On the 10<sup>th</sup> day, fresh weight, root, and shoot length of germinated seedlings were measured. Subsequently, the seedlings were placed in an oven at 70°C for 48 hours and dry weights were determined. Seedling fresh and dry weight was computed by adding the fresh and dry weights of root and shoot.

The seedling height is considered the sum of the length of the shoot and root; the seedling height stress index (SHSI) was calculated by the following formula:

$$SHSI = \frac{\text{Seedling height of stressed seedlings}}{\text{Seedling height of control seedling}} \times 100$$

Seed vigor index (SVI) was calculated as:

$$SVI = \frac{\text{Seedling dry weight (SDW)}}{\text{Mean germination time (MGT)}}$$

Water absorption rate (WAR) was calculated using the

following formula  $WAR (\%) = 100 (a-b)/b$ , where a is the weight (g) of seeds after soaking in distilled water for 24 h and b is the initial weight (g) of seed sample.

**Proline estimation:** Proline content from shoots was extracted according to the method of Bates et al. (1973).

**Determination of soluble sugars:** Total soluble sugars were determined by the phenol sulfuric acid method (Dubois et al., 1956) using glucose as standard.

**Statistical analysis:** Statistical analysis was performed using SAS program.

## RESULTS AND DISCUSSION

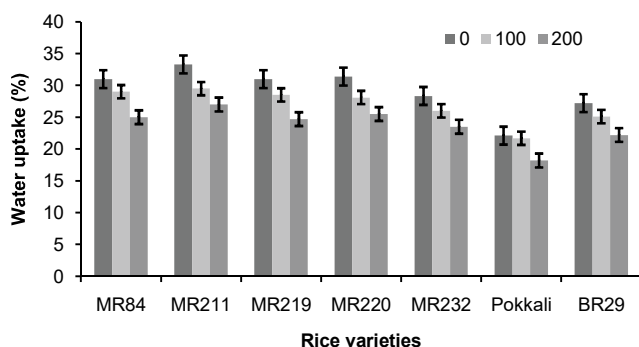
### Germination Percent (FGP), Germination Index (GI) and Germination rate:

In the control group, all cultivars exhibited 100% final germination. Up to a sodium chloride (NaCl) concentration of 100 mM, the final germination percentage remained consistent across all cultivars. However, notable differences emerged at extreme NaCl concentrations. The germination rates varied among cultivars at specific salt concentrations, with MR84, MR211, MR232, and Pokkali demonstrating the highest germination capability even at 100 mM NaCl. At 200 mM NaCl, there was a significant reduction in the final germination percentage for all cultivars. As NaCl levels increased, the germination index decreased, with a strong negative correlation coefficient between NaCl concentrations and the germination index. High-yielding cultivars demonstrated relatively consistent germination performance. Decline in gibberellic acid (GA) content in seeds due to salinity may contribute to reduced germination rates (Liu et al., 2018).

**Mean Germination Time (MGT):** In contrast, under normal conditions (control), germination started promptly with minimal variation in germination time. However, salinity caused delays in initiation and decreased the rate of germination, effects that became more pronounced with lower seed water content. The mean germination time (MGT) varied significantly among different treatments, gradually increasing with higher salt concentrations. Among Malaysian HYVs, MR84 exhibited the shortest MGT, statistically similar to other varieties. The escalating salt levels led to a decrease in MGT, indicating a slowdown in germination likely due to inhibited activation of enzymes crucial for reserve hydrolysis and mobilization. This pattern was consistent even at 100 mM. All cultivars managed to germinate under varying NaCl concentrations, the time taken differed based on cultivar and salt concentration. Higher NaCl levels primarily prolonged germination time rather than affecting the final germination percentage. Hence, MGT and germination index could serve as reliable indicators for assessing salt tolerance during germination, as salt-tolerant genotypes typically exhibit the shortest MGT and highest GI.

**Water uptake (%):** There was a clear relationship between water uptake and salinity, indicating that as salinity levels increase, water uptake decreases. Specifically, the increase in salinity stress significantly reduces water uptake. MR211 exhibits the highest water uptake, while Pokkali shows the lowest (Fig. 1). Variability in seed water uptake among rice cultivars is considerable, ranging from 22.14% to 33.33%, with significant differences. Moreover, water uptake is influenced by seed size. Despite differences in cultivars, their responses to salinity stress vary. Although there's no significant difference in water uptake as NaCl levels rise, seeds absorb water rapidly in the initial 6 hours. Throughout the measurement period, water uptake remains relatively consistent across NaCl levels. Imbibition, or water uptake, marks the initial stage of seed germination, wherein dry seeds, typically containing less than 10% water, absorb water and swell. This process kickstarts essential metabolic activities, including the breakdown of stored starches into sugars for energy and cellular structure formation.

**Root length:** MR219 showed the maximum root length under non-saline conditions, whereas MR232 had the



**Fig. 1.** Effect of NaCl on water uptake (%) of seeds of seven rice cultivars after placing in NaCl treatment at 0, 100 and 200 mM. Bars indicate standard errors

minimum (Table 2). However, this trend shifted at 100 mM and 200 mM NaCl concentrations. At 100 mM, MR84 displayed the longest root length (34.67 mm), accounting for 72.73% of the control, whereas MR232 and Pokkali had the shortest lengths. Root length of MR219, MR232, and MR220 was more affected compared to MR211, BR29, and Pokkali at higher salt concentrations indicating that the salt stress not only affected germination but also the growth of seedlings, which indicates that synthetic ability of seed and thus dry matter production of the seedlings was affected. This conforms with the findings of Vibhuti et al. (2015) where they observed that root length was conspicuously affected by salt.

**Shoot length:** The longest shoot length was observed in Pokkali, followed by MR219, which was statistically similar to other high-yielding Malaysian cultivars, while MR211 exhibited the minimum shoot length under normal conditions. Generally, shoot length decreased gradually as the NaCl concentration increased across all rice cultivars. At 200 mM NaCl, MR84 recorded the longest shoot length (19.18 mm), which accounted for 23.88% of the control. The decline in shoot length due to high salinity was minimal (34.64%) in MR211, while MR219 and MR220 showed the maximum decrease. This indicates that the shoot growth of MR220 was the most affected by NaCl levels.

**Seedling height:** Pokkali consistently produced the tallest seedlings, maintaining its superiority even in the presence of 100 mM NaCl. However, at 200 mM NaCl, there was a significant reduction in seedling height, down to 22% of the control level (Table 2). Except for MR211, there were no statistically significant differences in seedling height among the rice cultivars. MR211 produced the tallest seedlings at 200 mM NaCl, approximately 50% of the control level, followed by MR84 (Table 2). Both root and shoot lengths experienced substantial decreases with higher salinity levels. Root growth was more severely affected, decreasing

**Table 1.** Germination percentage, germination index, mean germination time and mean germination rate of some rice cultivars under different salinity levels

Cultivars	Germination (%)			Germination Index		Mean germination time			Mean germination rate		
	0 mM	100 mM	200 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
MR84	100.0a	100.0a	93.0ab	100.00a	93.00 ab	6.05d	6.44d	8.18b	37.53a	29.74ab	10.06bc
MR211	100.0a	100.0a	93.0ab	100.00a	93.00 ab	6.13d	6.52d	7.95b	35.68a-c	28.50ab	11.96ab
MR219	100.0a	95.0a	78.0abc	95.00a	78.00 bc	6.07d	6.50d	7.92b	36.98ab	27.08ab	9.88bc
MR220	100.0a	93.0a	87.0ab	93.00a	87.00 a-c	6.10d	6.53d	7.76b	36.32a-c	26.26b	12.46a
MR232	100.0a	100.0a	87.0ab	100.00a	87.00 a-c	6.26c	6.44d	7.97b	33.08c	30.04a	10.95ab
Pokkali	100.0a	98.0a	94.0a	98.00a	94.00 ab	6.17d	6.60c	8.31b	34.98a-c	26.53ab	8.11cd
BR29	100.0a	90.0a	73.0bc	90.00a	73.00 c	6.24d	6.86bc	8.23b	33.66bc	21.29c	6.98de
CV (%)	5.5	6.3	9.5	7.15	7.05	9.5	7.5	2.55	4.76	4.97	8.76

by up to 99% compared to a maximum reduction of 86% in shoot growth (Table 2). Conversely, in some species, root growth may remain unaffected or even increase under low salinity conditions, while shoot growth declines (Shelden and Munns 2023).

**Seedling height stress index (SHSI):** High salt levels consistently inhibited the growth of rice seedlings across different genetic varieties, as shown by the SHSI. The varieties MR84, MR211, and Pokkali demonstrated better growth at salt concentrations of 0-100 mM, while other varieties showed less favorable results. Some cultivars displayed mixed responses under salt concentrations of 100-200 mM.

**Seedling fresh weight:** The fresh weight of seedlings was consistently and significantly reduced in all rice cultivars at concentrations between 100 mM and 200 mM (Table 3). The shoot fresh weight of each cultivar fluctuated with varying NaCl concentrations. Seedling fresh weight varied from

38.33 to 92.67 mg. Among the rice cultivars, Pokkali had the highest seedling fresh weight, followed by MR220, which was statistically similar to MR84, MR219, and MR211.

**Timson index of germination velocity:** Salinity gradually reduced the germination velocity (Timson index). Values of the Timson index did not differ significantly among the rice genotypes in the control (Table 3). At 100 mM NaCl, the highest Timson index was recorded in MR232 which is comparable to MR84 and MR211 while the minimum was recorded in BR29. However, this trend was different at 200 mM NaCl where MR220 possessed the highest value.

**Seed vigour index (SVI):** Seed vigour index differed significantly among the rice genotypes even in the control environment (Table 3). Rice genotypes responded differently to different salt levels. The SVI sharply decreased as the NaCl levels increased and it was maximum in Pokkali followed by MR220 which was statistically similar to MR84

**Table 2.** Effect of different NaCl levels on shoot and root length, seedling height and seedling height stress index of rice cultivars.

Cultivars	Shoot length (mm)			Root length (mm)			Seedling height (mm)			SHTI	
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	100 mM	200 mM
MR84	80.33b (100)	22.31c-e (27.78)	19.18a (23.88)	47.67c (100)	34.67a (72.73)	0.67cd (1.40)	128.00b	56.98ab	19.85b	44.52a	15.50bc
MR211	52.00c (100)	25.29c (48.63)	18.01ab (34.64)	45.33cd (100)	16.00c (35.29)	2.68a (5.90)	97.33d	34.01c	27.96a	35.15bc	29.08a
MR219	84.67b (100)	20.98cd (24.78)	13.39d (15.82)	66.00a (100)	14.33cd (21.72)	0.50cd (0.76)	150.67a	35.31c	13.89cd	23.51d	9.27d
MR220	84.33b (100)	33.02b (39.16)	13.19d (15.64)	41.00d (100)	21.00b (51.22)	0.83c (2.03)	125.33b	54.02b	14.02cd	43.31ab	11.18d
MR232	80.67b (100)	21.00cd (26.03)	17.50ab (21.70)	28.33f (100)	12.33cd (43.53)	0.50cd (1.76)	109.00c	33.33c	18.00bc	30.58cd	16.54b
Pokkali	103.67a (100)	56.46a (54.46)	17.26a-c (16.65)	56.33b (100)	12.02cd (21.34)	2.17b (3.85)	160.00a	68.48a	19.43b	42.92ab	12.13cd
BR29	72.33b (100)	25.00c (34.56)	16.00b-d (22.12)	38.33e (100)	15.09c (39.37)	2.00b (5.22)	110.67c	40.09c	18.00bc	36.20a-c	16.27bc
CV (%)	7.2	9.42	7.18	8.0	10.0	9.0	3.2	9.9	9.5	9.4	9.8

SHTI- Seedling height stress index; Values in parentheses represent the percentage relative to untreated control plants (set at 100%)

**Table 3.** Seedling fresh weight, Timson index of germination velocity and Seed vigor index of rice cultivars

Cultivars	Seedling fresh weight (mg)			Timson index of germination velocity			Seed vigor index (SVI)		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
MR84	62.00b	40.67b	21.60bc	49.17	41.93	22.47	2.31b	0.88ab	0.45bcd
MR211	52.33c	26.00de	26.03a-c	47.33	41.37	25.37	2.09bc	0.65cd	0.34de
MR219	64.67b	30.97de	24.67a-c	48.73	38.83	20.13	1.86c	0.77bc	0.39cde
MR220	65.00b	39.50bc	20.83cd	48.17	37.37	26.03	2.31b	0.80bc	0.52b
MR232	52.33c	27.33de	19.93cd	45.17	42.50	22.73	1.86c	0.80bc	0.48bc
Pokkali	92.67a	58.23a	29.57a	46.73	39.40	19.30	3.35a	0.98a	0.71a
BR29	48.33cd	24.00e	21.33b-d	45.73	35.43	15.10	1.33d	0.70cd	0.55b
CV (%)	4.54	8.57	11.17	5.5	7.2	4.5	6.9	7.75	8.61

**Table 4.** Effect of NaCl on proline and sugar content in rice seedlings

Cultivars	Proline			Sugar content		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
MR84	4.41±0.09	3.42±0.58	2.76±0.14	43.0±2.11	51.20±2.11	28.35±0.03
MR211	1.03±0.16	1.59±0.04	1.53±0.34	36.21±2.72	43.04±0.33	20.81±0.37
MR219	6.33±0.29	7.17±0.63	1.18±0.01	60.88±0.57	54.07±5.36	21.81±1.05
MR220	4.49±0.07	2.63±0.25	2.82±0.39	76.68±10.51	50.62±5.92	30.99±9.15
MR232	3.75±0.51	4.95±0.045	2.67±0.28	59.74±6.36	71.35±9.84	46.16±2.99
Pokkali	1.53±0.12	1.88±0.01	1.33±0.11	64.89±3.15	55.18±6.68	24.95±1.70
BR29	45.73	35.43	15.10	1.33d	0.70cd	0.55b
CV (%)	5.5	7.2	4.5	6.9	7.75	8.61

and MR211 while it was minimum in BR29.

**Proline content:** The amount of proline substantially increased in plants under salt stress and generally followed an increasing trend except for MR84 and MR232 (Table 4). There was a significant variation in proline content among all rice cultivars. MR211 and MR185 genotypes accumulated the maximum amount of proline while BR29 and MR220 genotypes had the minimum amount of proline in their shoot tissue. MR 211 cultivar showed high amounts of proline in both salt-stressed and control treatments and is also tolerant to salinity stress (Table 4). These results are similar to Danai-Tambhale et al. (2011) but contradictory to Momayezi et al. (2009). Momayezi et al. (2009) have reported that the highest accumulation of proline was detected at a salt concentration of 5 dS m<sup>-1</sup> and a decreasing tendency was noted beyond this point. Moreover, there was no proven correlation found between the growth metrics and proline accumulation (Momayezi et al., 2009). The seed priming with varying concentrations of proline significantly enhanced the germination (%), seed vigour index, and  $\alpha$ -amylase activity of rice genotypes under both normal and salinity conditions (Singh et al., 2018). The beneficial effect of seed priming with proline on various traits was more pronounced under salinity than in normal conditions.

**Sugar content:** The salinity stress increased sugar content in MR211 and MR232, while sugar content was decreased in MR84, MR219, MR220, Pokkali, and BR29 (Table 4). The total sugar in the treated seedlings was reduced to 48%. The accumulation of soluble sugars in response to salinity is quite well documented in many plant species (Dubey and Singh 1999, Pattanagul and Thitisaksakul 2008). While the rise in sugar buildup in reaction to salinity stress was only observed in salt-sensitive cultivars (Pattanagul and Thitisaksakul 2008), it was also observed in MR211, a cultivar that is moderately tolerant to saline conditions (Table 4). The under salinity stress accumulation of sugars along with other compatible solutes contributes to an osmotic adjustment

allowing the plants to maximize sufficient storage reserves to support basal metabolism under a stressed environment (Dubey and Singh 1999). Soluble sugars may function as a typical osmo protectant, stabilizing cellular membranes and maintaining turgor pressure.

## CONCLUSION

Rice has higher salt tolerance during the seed germination phase compared to the initial seedling stage, like other crops like barley, wheat, and triticale. Contemporary rice varieties, except BR29, show higher salt tolerance during the early stages. Evaluating salt resistance during advanced growth phases is crucial for accurate rice cultivars, which is currently underway in our research.

## AUTHOR'S CONTRIBUTION

All authors contributed equally to the conception, execution, data collection, data analysis, writing, and critically revised the manuscript, and approved the final version.

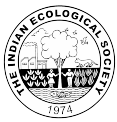
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Received 05 October, 2024; Accepted 10 January, 2025



# Elemental Composition of Soil Primary Particles in *Alfisols* of Northern Karnataka

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**Abstract:** Study on total elemental composition of soil primary particles in Bailmadapura micro-watershed of Gadag district, Karnataka was conducted during 2022-23. Horizon-wise soil samples were collected and analysed for major and trace elemental content in sand, silt and clay. *Alfisol* pedons 1 and 2 revealed the various patterns in elemental composition across horizons and soil fractions. Elemental analysis showed significant variations in the composition of major elements (Si, Al, Fe, Ca, Mg and K) and trace elements (Zn, Pb, Sr and Cu) among soil fractions (sand, silt, clay) at different depths. In Pedon 1, the upper horizon (Ap) showed higher concentrations of Si, Al and Fe in the sand fraction, with erratic distribution of Ca, Mg, P and S. The Bt1 horizon exhibited changes in element content, particularly in Fe concentration and the distribution of trace elements. The Bt2 horizon displayed further alterations, with Si and Al maintaining consistent patterns, while trace elements exhibited different distributions influenced by soil forming processes. In Pedon 2, similar trends were observed, emphasizing the influence of soil fraction and depth on elemental composition. The findings highlighted the complexity of interactions involving mineralogical composition, organic matter content, redox conditions, and pH in shaping elemental distribution in *Alfisols*.

**Keywords:** Alfisols sand, Silt, Clay, Soil primary particles

The total elemental composition of soil primary particles in *Alfisols* could be complex and highly variable, depending on the landscape and environmental conditions. Total major and trace elemental composition in virgin soil generally depends on geochemical, geomorphic and pedological processes and the lithology of the soil parent material. The elemental composition of soil particles is influenced by weathering of parent material, secondary mineral formation, organic matter decomposition and leaching. The breakdown of rocks and minerals in the parent material through weathering releases various elements into the soil. Organic matter decomposition contributes to the release of carbon, nitrogen and other nutrients. Leaching, the movement of water through the soil profile can lead to the loss of certain elements, especially in areas with high rainfall.

The total elemental composition of soil particles and bulk soil will allow a better understanding of the presence of major and trace elements in available form (Sanjay and Kuligod, 2020). In this context, the information on the total elemental composition of soils is essential not only for the evaluation of inherent soil fertility and soil quality from an agricultural point of view but also for the understanding of the stock and flow of elements through a variety of natural and anthropogenic processes. Vertical distribution of major and trace elements in soil particles gets affected by plant cycling relative to leaching, weathering, dissolution and atmospheric deposition. As the interface between the atmosphere, biosphere and lithosphere, soil undergoes an intense vertical

exchange of materials resulting in steep chemical and physical gradients from surface to bedrock. The present study helps to understand pedological evolution of *Alfisols* of Northern Karnataka.

## MATERIAL AND METHODS

Bailmadapura MWS in Shirahatti taluk, Gadag district is located between 14° 59'-15° 10' North latitudes and 75° 34'-75° 37' East longitudes. Bailmadapura MWS comes under Agro-climatic Zone 3: Northern Dry Zone. Most of the MWS is at an elevation of 450-800 m above MSL. The average annual rainfall of the zone ranges from 464 to 785 mm. The soils are medium red soils in major areas, with sand loams in the remaining areas. The main cropping season is *Rabi*. Groundnut, chickpea, wheat, sorghum and sugarcane are the important crops of the zone.

The samples were collected from each master and sub-horizons of soil profiles and their morphological characters were recorded by describing the profile. Collected soil samples were dried in shade. The air-dried samples were ground with a wooden pestle and mortar and passed through a 2 mm sieve to separate the coarse fragments (> 2 mm). Particle size-distribution of soil samples was determined by international pipette method using sodium hexametaphosphate as a dispersing agent for separation of sand, silt and clay (Piper 2002). Each soil fractions. (0.25g) were subjected for microwave digestion with concentrated nitric and hydrofluoric acids at 220° C for 2 hours and



immediate cooling for >30 minutes (Silva et al., 2016). The digested solution was filtered by grade 40 paper and stored in polyethylene bottle and kept in refrigerator. Total content of major elements was determined by feeding extractant to ICP-OES (inductively coupled plasma optical emission spectroscopy), AAS (atomic absorption spectroscopy), flame photometer and spectroscopy for total silicon and aluminium, total iron and manganese, potassium and phosphorus and sulphur, respectively. The total calcium and magnesium content were determined by adopting Versenate titration method. The experimental data was subjected to statistical analysis by using SPSS software (2002).

## RESULTS AND DISCUSSION

**Total elemental composition of pedon 1 of Alfisol:** The comprehensive overview of the distribution of major and trace elements within the pedons of *Alfisol* is presented across different horizons, depths and soil fractions.

In pedon 1 across the various studied horizons (Ap, Bt1 and Bt2) there variation in the elemental composition (Table 1). The soil fractions sand, silt and clay exhibited differences in their elemental composition. This is expected since each soil fraction has distinct physical and chemical properties, which affect the retention and molecular availability of different fractions. Total elemental composition varied across different soil horizons. This is attributed to differences in the processes that occur at different soil depths. In the uppermost horizon (Ap), the major elements Si, Al and Fe were significantly higher (110.92, 54.93 and 52.39 g kg<sup>-1</sup>) in sand fractions and maintained relatively consistent and higher concentrations across the silt and clay soil fractions, indicating their widespread presence throughout the soil. The Ca, Mg, P and S concentrations showed an erratic distribution across the soil fractions. Trace elements like Zn, Pb and Sr (97.30, 18.22 and 56.83 mg kg<sup>-1</sup> respectively) exhibited diverse behaviour, with their concentrations varying between sand, silt and clay fractions. As the depth increased into the Bt1 horizon, there were changes in element content in different fractions. The major elements Si and Al (129.25 and 57.28 g kg<sup>-1</sup>) continued to show consistent patterns, while Fe concentrations decline. The concentrations of Ca, Mg, P and S was lower in sand content. Trace elements, on the other hand, continued to display variability. Zn and Pb concentrations increased in the clay fraction. Similarly, Cu concentration rose in the clay fraction (79.67 mg kg<sup>-1</sup>), indicating a distinct distribution pattern. Trace elements (Zn, Pb and Sr) recorded variable behaviour across different fractions, indicating complex interactions. This might have resulted from factors such as mineral adsorption, organic matter content, mineralogical

**Table 1.** Distribution of elements (major and trace) in soil fractions of pedon 1 of *Alfisol*

Horizon	Soil fraction	Major elements (g kg <sup>-1</sup> )													Trace elements (mg kg <sup>-1</sup> )									
		Si	Al	Fe	K	Ca	Mg	P	S	Mn	Zn	Pb	Sr	Cr	Cu	V	Ni	Co	Rb	As				
Ap	Sand	110.92	54.93	52.39	4.52	5.34	2.48	1.19	0.83	1.79	69.40	13.30	42.58	38.71	51.76	32.80	25.15	14.01	5.19	3.38				
	Silt	96.22	63.22	75.27	5.66	8.03	4.55	1.99	1.27	2.65	124.30	19.74	63.90	58.09	87.09	54.19	44.61	25.11	11.31	Tr				
	Clay	97.73	41.27	49.68	3.96	5.98	5.65	1.19	1.15	2.75	98.21	21.62	64.01	58.19	71.12	47.88	42.36	24.86	10.48	Tr				
	Mean	101.62	53.14	59.11	4.71	6.45	4.23	1.46	1.08	2.40	97.30	18.22	56.83	51.66	69.99	44.96	37.37	21.33	8.99	3.38				
Bt1	Sand	157.71	48.28	44.96	3.96	5.98	3.58	1.65	0.57	1.23	31.74	8.41	28.74	26.13	29.99	22.07	18.13	10.29	4.04	2.11				
	Silt	117.42	56.45	55.90	4.56	6.96	5.48	2.49	0.88	1.73	58.16	13.01	41.92	38.11	41.85	30.73	25.99	14.75	5.99	Tr				
	Clay	112.61	67.12	65.80	6.51	9.14	6.75	3.13	1.63	3.24	96.74	20.36	76.11	69.19	79.67	56.51	50.74	30.91	12.29	Tr				
	Mean	129.25	57.28	55.55	5.01	7.36	5.27	2.42	1.03	2.07	62.21	13.93	48.92	44.48	50.50	36.44	31.62	18.65	7.44	2.11				
Bt2	Sand	137.08	32.38	29.14	4.98	5.33	2.32	2.17	1.77	2.03	23.88	7.20	22.45	20.53	19.95	18.34	13.62	7.84	3.76	3.45				
	Silt	103.62	36.78	36.55	5.84	6.19	2.68	2.59	2.04	2.37	38.59	10.05	32.55	29.71	29.02	27.54	20.33	12.41	5.41	Tr				
	Clay	88.19	40.88	51.00	4.55	7.60	3.41	3.40	2.33	2.67	59.99	12.82	52.30	47.66	46.62	43.92	37.74	21.67	12.00	Tr				
	Mean	109.63	36.68	38.90	5.12	6.37	2.80	2.72	2.05	2.36	40.82	10.02	35.77	32.63	31.86	29.93	23.90	13.97	7.06	3.45				

Tr- Traces

composition and redox conditions in the soil. These findings were in conformity with Liu et al. (2002). The Bt2 horizon, representing deeper depths, showcased further changes in elemental content. Major elements Si, Al, Fe, Ca, Mg and K maintained consistent trends, while trace elements like Zn and Pb exhibited slightly different patterns compared to the shallower horizons. The mean concentration of total elemental composition indicated the erratic distribution of major and trace elements across the soil depth. In Bt2 horizon, major elements like Si and Al continued to display consistent and higher concentrations, while Fe remained lower than in the upper horizons due to mineralogical composition and weathering process affecting this element. Soil forming process transformation were influencing the distribution of these elements across different depths. This is typical as iron oxides are prone to reduction and leaching at greater depths. Similar distribution pattern and mobility of elements was observed by Dey (1999) in *Alfisols*. Trace elements (Zn and Pb) exhibited different patterns of content at deeper depth compared to shallower horizons. This kind of distribution was influenced by changes in pH, redox conditions, mineralogical content and the availability of colloids for adsorption

**Total elemental composition of pedon 2 of *Alfisol*:** In pedon 2, across the horizons (Ap, Bt1 and Bt2) there was a variation in the elemental composition (Table 2). In the uppermost horizon (Ap), the major elements Si, Al and Fe (101.62, 53.14 and 59.11 g kg<sup>-1</sup> respectively) displayed consistent patterns across soil fractions, suggesting their prevalence throughout the soil. Trace elements such as Zn, Pb and Sr (97.30, 18.22 and 56.83 g kg<sup>-1</sup>, respectively) exhibited diverse behaviour, with varying concentrations among the fractions. Major elements (Si, Al and Fe) were significantly higher in the sand fraction of *Alfisols*. This pattern revealed that these major elements were prevalent in the coarser and primary mineral soil particles and are relatively consistent throughout the soil profile. This could be due to the presence of minerals like quartz (Si) and iron oxides (Fe) in the sand fraction. In the Bt1 horizon, there were changes in element concentration emerge. The major elements Si and Al maintained their consistent higher content, while Fe concentration showed a decrease. Concentration of Ca, Mg, P and S was lower in sand fraction. Trace elements exhibited intricate trends; Zn and Pb concentrations rose in the clay fraction. Cu concentration increased in the clay fraction, revealing distinct spatial patterns. Arsenic concentration was present in traces in silt and clay fractions across all the horizons. In Bt1 horizon major elements such as Si and Al maintained consistent patterns of distribution, while Fe concentrations declined.

**Table 2.** Distribution of elements (major and trace) in soil fractions of pedon 2 of *Alfisol*

Horizon	Soil fraction	Major elements (g kg <sup>-1</sup> )											Trace elements (mg kg <sup>-1</sup> )										
		Si	Al	Fe	K	Ca	Mg	P	S	Mn	Zn	Pb	Sr	Cr	Cu	V	Ni	Co	Rb	As			
Ap	Sand	156.86	35.17	40.23	5.35	6.18	5.51	3.16	2.83	3.71	38.72	12.14	30.03	27.51	30.92	22.01	18.02	10.72	5.7	0.32			
	Silt	108.81	37.59	49.25	5.97	7.43	6.12	3.17	3.16	4.18	71.79	16.6	44.45	40.62	45.91	30.98	25.34	14.91	7.67	Tr			
	Clay	95.21	45.48	64.03	6.6	9.28	7.11	3.43	3.39	5.13	104.77	15.14	66.25	60.44	74.68	48.44	43.75	28.03	13.75	Tr			
Bt1	Mean	120.29	39.41	51.17	5.97	7.63	6.25	3.25	3.13	4.34	71.76	14.63	46.91	42.86	50.50	33.81	29.04	17.89	9.04	0.32			
	Sand	127.38	40.14	32.71	6.4	8.14	5.01	4.04	3.91	4.51	36.12	10.02	24.22	22.33	22.87	18.85	15.55	10.46	6.83	4.58			
	Silt	112.54	40.9	32.54	6.96	9.87	6.13	6.08	5.25	6.24	51.65	11.51	46.48	35.48	27.89	29.27	22	15.03	9.81	Tr			
Bt2	Clay	90.24	51.94	38.94	6.28	9.99	5.83	4.3	4.58	4.78	65.87	17.38	56.18	51.39	48.96	44.94	39.74	22.7	15.18	Tr			
	Mean	110.05	44.33	34.73	6.55	9.33	5.66	4.81	4.58	5.18	51.21	12.97	42.29	36.40	33.24	31.02	25.76	16.06	10.61	4.58			
	Sand	123.93	36.69	29.26	2.95	4.69	1.56	0.59	0.46	1.06	32.67	6.57	20.77	18.88	19.42	15.4	12.1	7.01	3.38	1.13			
Tr- Traces	Silt	109.09	37.45	29.09	3.51	6.42	2.68	2.63	1.8	2.79	48.2	8.06	43.03	32.03	24.44	25.82	18.55	11.58	6.36	Tr			
	Clay	86.79	48.49	35.49	2.83	6.54	2.38	0.85	1.13	1.33	62.42	13.93	52.73	47.94	45.51	41.49	36.29	19.25	11.73	Tr			
	Mean	106.60	40.88	31.28	3.10	5.88	2.21	1.36	1.13	1.73	47.76	9.52	38.84	32.95	29.79	27.57	22.31	12.61	7.16	1.13			

This decline in Fe might be due to the reduction of iron oxides as one moves deeper into the soil profile. Trace elements (Zn, Pb and Cu) concentrations increased in the clay fraction, revealing occlusion and co-precipitation. This could be attributed to large surface area for adsorption and retention. Alekseeva et al. (2011) also made similar observation. Transitioning to the Bt2 horizon at greater depths, further shifts in element distribution become apparent. Major elements Si, Al, Fe, K, Ca and Mg continued to exhibit consistent and higher content, while trace elements such as Zn and Pb manifested distinct patterns compared to shallower horizons.

### CONCLUSION

This study provides valuable insights into the total elemental composition of *Alfisols*, emphasizing the influence of soil depth and fractions on major and trace element distribution. The consistent patterns observed in major elements (Si, Al and Fe) across soil horizons and fractions indicate their prevalence in specific soil fractions, likely associated with primary minerals such as quartz and iron oxides. The variations in trace element distribution suggest intricate interactions influenced by factors like pH, redox conditions, and mineralogical content. The decline in Fe concentration with depth in both pedons indicated potential reduction of iron oxides. The study contributes to understanding the dynamic nature of elemental composition

in *Alfisols*, providing essential knowledge for soil management and environmental studies.

### ACKNOWLEDGMENT

The author is thankful to 'The Karnataka Science and Technology Promotion Society (KSTEPS), Department of Science and Technology, Govt. of Karnataka with grant no AGR-13:2022-23/981 for providing financial assistance in the form of a stipend during the course of the investigation.

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# Effect of Integrated Nutrient Management on Growth and Yield of Wheat (*Triticum aestivum* L.) in Sandy Soil of West Bengal

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**Abstract:** The influence of nano urea as foliar spray along with split application of potassium was studied on wheat cv. DBW 187 (Karan Vandana) during 2022-2023 at Seacom Skills University, Birbhum, West Bengal. The maximum plant height, more dry matter production, more number of tillers per m<sup>2</sup> were observed in treatment with Basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% Nano Urea and 1% MOP solution at Crown root initiation and Flag Leaf stage. The same treatment showed highest number of spikes/m<sup>2</sup>, spike length, filled grains/spike, test weight, grain yield and straw yield as well. Soil enzymatic activity was maximum in RDF with split potassium doses. The gross returns, net returns and benefit: cost ratio too were maximum in the same treatment. Hence, a basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% Nano Urea and 1% MOP solution at CRI and Flag Leaf stage was beneficial for wheat production in the Red and Lateritic soils of West Bengal, keeping soil health in view.

**Keywords:** Wheat, Nano urea, Enzymatic activity, Potassium

Wheat (*Triticum aestivum* L.) accounts for approximately one-fifth of the total area under food grains and provides roughly one-third (40%) of India's total food grain production, showcasing the significant role of this crop in India's food security (Pyne et al., 2022). India is the second largest producer of wheat, accounting 12 per cent of the global production (Kundu et al., 2020). West Bengal produces only a small fraction of India's total wheat output, accounting for 5.15% of the state's food grain production and 3.1% of the national production. Given the region's population growth and dietary preferences, it is imperative to increase wheat productivity in West Bengal to satisfy the growing food demands (Goswami et al., 2020). Following the introduction of the Green Revolution in India, food production soared, paralleled by a rapid escalation in the use of chemical fertilizers, which in turn has triggered a range of environmental concerns and negative impacts on soil health. While fertilizer is necessary in producing high yields, synthetic fertilizers have environmental consequences, such as altering the global nutrient budget, affecting water and air quality, and ultimately contributing to climate change through greenhouse gas emissions (Lu 2017). Even nutrients applied through the fertilizers at the time of sowing are not fully utilized by the crop and are lost through various ways (leaching, fixation etc.) and the crop may suffer from nutrients deficiency at the later stage (Pratihari et al., 2023). Despite decades of technological advances, the efficiency of N, P, and K fertilizers has remained relatively constant at 30-35, 15-20, and 35-40%, respectively,

with a substantial proportion of the applied fertilizer accumulating in the soil or leaching into aquatic systems, contributing to eutrophication (Yahaya 2023). All these led to major issue through which farmers have been facing two simultaneous challenges i.e., to cater to the ever increasing population and avoiding inorganics on soil. Thus, by decreasing its soil requirement of inorganic could be curtailed to some extent, through foliar application to compensate the nutrient requirement. Foliar spray of one or more nutrients to supplement soil application of fertilizers has been gaining more attention in recent years to overcome the problem of low fertilizer nutrient supply from soil to plant (Verma et al 2023). NPK 19:19:19 fertilizer is available as hundred percent water soluble complete fertilizer containing nitrogen, phosphorus and potassium each with 19% low salt index. Nano fertilizers have distinctive novel characteristics than the bulk materials and also have enormous advantages over the traditional fertilizers and used to improvise the crop production and soil properties in general (Roy et al., 2023). Nano Urea (Nano Nitrogen), developed by Indian Farmers Fertilizer Cooperative Limited (IFFCO), provides a novel alternative to ease farmers away from urea. Foliar fertilizers can provide the plant nutrient at critical stages of plant growth when the nutrient requirement of plant exceeds the normal uptake for certain nutrients (Sarkar et al., 2021). Large amount of potassium is required by wheat, and once the reproduction stage is finished, a constant supply is required until heading (Sharma et al., 2022). The present study was conducted to

find out the best possible application of liquid fertilizers NPK 19:19:19 and nano urea comparing with the conventional soil application of recommended dose of fertilizer.

### MATERIAL AND METHODS

Field experiment was undertaken in wheat in *Rabi* season of 2022-23 at the Instructional Farm (23°70' E latitude, 87°67' N longitude and 9.75 m above mean sea level) of Seacom Skills University, Kendradangal, Birbhum, West Bengal, India to observe if substituting soil application of inorganics (either partly or fully) with foliar spray could be possible without sacrificing the production and degradation of soil health. The soil of experimental field was sandy loam soil, with slightly acidic (pH 5.9), available N (186 kg/ha), phosphorus (16.37 kg/ha) and potassium (225.06 kg/ha). The trial was conducted in a randomised block design with 3 replications consisting of 8 different treatments with a plot size of 6.0 X 4.0 m<sup>2</sup> (Table 1). The biofortified, high yielding variety of wheat [DBW 187 (Karan Vandana)] was line sown on 20<sup>th</sup> November, 2022 and harvested on 10<sup>th</sup> March, 2023. The inter row spacing given was 22.5 cm with a depth of 4 cm to get an uniform germination. The growth parameters, yield and yield attributes were recorded at specified stages with standard procedure. After maturity, harvested plant samples were collected, oven dried and ground for analyzed to study the total uptake of N, P and K following the standard procedures (Jackson 1973), soil enzymatic activity like dehydrogenase activity (DHA), acid phosphatase activity (APA) were determined (Sahoo et al 2022). The Cost of cultivation were estimated and gross and net return (as per market price in West Bengal) were worked out for economic analysis. The experimental data were analyzed by SPSS (Version-26.0) package. The comparison of treatments was made with or Duncan's Multiple Range Test (DMRT).

### RESULTS AND DISCUSSION

**Growth parameters:** The treatment comprises of basal

application of 50:60:20 kg NPK/ha and foliar spray with 0.5% Nano Urea and 1% MOP solution at CRI and flag leaf stage attained the highest plant height (76.12 cm) with more number of tillers per m<sup>2</sup> (370.93) (Table 2). This treatment also showed highest leaf area index (LAI) (4.94) and dry matter production (910.7 g/m<sup>2</sup>) closely followed by the treatment T<sub>4</sub>- Basal dose of 50:60:20 kg NPK/ha + top dressing of 25:20 kg NK/ha at CRI stage and 25 kg N/ha at flag leaf stage (RDF with split application of N and K as well) (4.91) and (891.2 g /m<sup>2</sup>). It might be due to increased photosynthetic rate and higher leaf area that increased total dry matter production observed by supplementation of nitrogen through foliar spray (Rawate et al 2022). Moreover, foliar nutrient of MOP along with nano urea, produced an optimal nutrient supply and, as a result, enhanced meristematic cell activity and cell elongation, both of which are known to have positive impacts on the metabolic process and improved vegetative development (Patel et al 2022). In addition, the rate of growth was also highest (17.52) in T<sub>6</sub>- Basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% Nano Urea and 1% MOP solution at CRI and Flag Leaf stage, which is because of ample nitrogen supply as well as availability, generating an optimal environment for root zone growth and development. The results were in conformity to the findings of Choudhary et al. (2023).

**Yield attributes and yield:** Basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% nano urea and 1% MOP solution at CRI and Flag Leaf stage has shown a hike in yield attributes and yield of wheat, compared to control treatment (T<sub>1</sub>) as well as only application of RDF (Table 2). The number of spikes/m<sup>2</sup> varied between 119.56 and 295.67. Highest number of spikes/m<sup>2</sup> (295.67) was in T<sub>6</sub>- Basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% Nano Urea and 1% MOP solution at CRI and flag leaf stage. Similar trend was also observed in spike length (14.04 cm). The increase was because of positive response in terms of availability of nutrients through foliar application of nano urea as well as split

**Table 1.** Treatment details

Sl. no.	Treatment details
T <sub>1</sub>	Absolute control (no NPK)
T <sub>2</sub>	Basal dose of 50:60:40 kg NPK/ha + top dressing of 25 kg N/ha at CRI and Flag Leaf stage {RDF (conventional application)}
T <sub>3</sub>	Basal dose of 25:30:20 kg NPK/ha + top dressing of 12.5 kg N/ha at CRI and Flag Leaf stage (50% RDF)
T <sub>4</sub>	Basal dose of 50:60:20 kg NPK/ha + top dressing of 25:20 kg NK/ha at CRI stage and 25 kg N/ha at Flag Leaf stage (RDF with split application of N and K as well)
T <sub>5</sub>	Only foliar spray with 0.5% solution of 19:19:19 NPK at 10 Days interval, starting from 11 DAS
T <sub>6</sub>	Basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% Nano Urea and 1% MOP solution at CRI and Flag Leaf stage
T <sub>7</sub>	Basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% solution of 19:19:19 NPK at CRI and Flag Leaf stage
T <sub>8</sub>	Basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% solution of Nano Urea at CRI and Flag Leaf stage

doses of potassium through MOP. Similar kind of results were demonstrated by other investigator (Rawate et al 2022). This also led to increased number of filled grains/spike which varied from 8.56 to 27.82 with an average increase of 225% in the treatment  $T_6$  as compared with  $T_1$  (absolute control). Test weight varied from 37.36 to 40.31 g with variation of 7.9% and was highest in treatment  $T_6$ . The grain yield varied between 1660 to 4292 kg/ha and highest value was recorded from  $T_6$  treatment which was statistically at par and closely followed by treatment  $T_4$  and lowest grain yield of wheat was in control treatment. Wheat straw yield also followed the same trend, varied from 3824 to 6262 kg/ha and highest value observed was in  $T_6$ . The synergistic effect of the integrated use of potassium in splits (basal and foliar) along with nano urea might have improved several yield-contributing traits, as well as various vegetative growth characteristics, enhancing the overall performance of the crop. Nano fertilizers have been found to enhance the efficiency of conventional fertilizers by facilitating better absorption of nutrients through plant cells, resulting in optimal growth of plant parts and metabolic processes, such as photosynthesis. This led to increased accumulation and translocation of photosynthate to the economic parts of the plant, thereby enhancing source and sink strength and ultimately resulting in higher yield (Ojha et al., 2023, Patel et al., 2022). The harvest index of wheat increased from 30.89 to 41.30 and the hike was noted up to 33.70%. Highest value was recorded in the treatment ( $T_6$ ). The foliar application of nitrogen in combination with split doses of potassium led to proper maintenance of leaf nutrients and enhanced photosynthetic capacity, ultimately leading to increased grain and stover yield. The improved photosynthate formation, as a result of the foliar application of nano urea, led to a significant positive impact on the harvest index.

**Soil enzymatic activity:** Dhydrogenase activity varied from 9.2 to 11.3 ( $\mu\text{g TPF/gm/24 hr}$ ) with a variation of 22.82% and acid phosphatase activity varied from 23.18 to 28.69 ( $\mu\text{g PNPP/gm/ hr}$ ) with an average rise of 23.77% (Table 3). In both cases highest activity was recorded in  $T_4$  treatment. Split doses of nitrogen and potassium led to better availability of nutrients to plants might have resulted in higher uptake of them through which less disturbance occurred on soil biotics elements and in fact application of foliar spray of nano urea in  $T_6$  plot has also shown significant effect on the microbial activity in soil which led to almost equivalent result of dehydrogenase activity as well as acid phosphatase, giving a hike in yield of wheat. This results were also observed on pea by Symanowicz et al. (2018).

**Nutrient uptake:** After the harvest, nutrient uptake of grain and straw varied significantly with different split nutrient management practices as per treatments (Table 3). The total nitrogen uptake varied from 43.6 to 118.6 kg/ha, with the variation being 172.02%. Phosphorus uptake varied from 5.57 to 16.22 kg/ha, with the variation of 191.20% and the potassium uptake varied from 49.6 to 114.1 kg/ha and the variation was 130.04%. The foliar application of nano-fertilizers might have allowed the rapid absorption of nutrients through the epidermis or stomata, followed by efficient translocation via apoplast or symplast pathways, significantly improving the nitrogen uptake of the plants (Kumar et al., 2023). Furthermore, as only half the dose of urea was applied as basal fertilizer, it was completely absorbed by the plants due to their high nitrogen demand during vegetative growth, thus reducing nitrogen loss. The reduced urea application also allowed beneficial biotic colonies to mobilize nutrients such as phosphorus, resulting in higher uptake of this nutrient. Even the split doses of

**Table 2.** Effect of integrated nutrient management on growth and yield of wheat

Treatment	Plant height (cm) at harvest	Dry matter production ( $\text{g/m}^2$ ) at harvest	No. of tillers/ $\text{m}^2$ at harvest	LAI (90 DAS)	CGR (61-90)	No. of spikes/ $\text{m}^2$	Length of spikes (cm)	Number of filled grains/spike	Test weight (g)	Grain yield (kg/ha)	Straw yield (kg/ha)	Harvest index (%)
$T_1$	45.59 <sup>a</sup>	443.6 <sup>d</sup>	168.59 <sup>e</sup>	1.86 <sup>d</sup>	7.72 <sup>c</sup>	119.56 <sup>d</sup>	9.55 <sup>a</sup>	8.56 <sup>c</sup>	37.36	1660 <sup>b</sup>	3824 <sup>b</sup>	30.89 <sup>b</sup>
$T_2$	70.10 <sup>a,b</sup>	891.0 <sup>a</sup>	327.02 <sup>b,c</sup>	4.55 <sup>a</sup>	17.73 <sup>a</sup>	252.23 <sup>b,c</sup>	12.09 <sup>b</sup>	19.35 <sup>b</sup>	39.86	3909 <sup>a,b</sup>	5812 <sup>a</sup>	40.22 <sup>a</sup>
$T_3$	56.43 <sup>d</sup>	551.2 <sup>c</sup>	265.25 <sup>d</sup>	3.50 <sup>b,c</sup>	8.73 <sup>c</sup>	222.97 <sup>c</sup>	10.43 <sup>c,d,e</sup>	17.95 <sup>b</sup>	39.54	3468 <sup>c,d</sup>	5800 <sup>a</sup>	37.48 <sup>a</sup>
$T_4$	72.05 <sup>a</sup>	891.2 <sup>a</sup>	354.63 <sup>a,b</sup>	4.91 <sup>a</sup>	17.59 <sup>a</sup>	284.82 <sup>a</sup>	12.63 <sup>b</sup>	27.6 <sup>a</sup>	40.28	4011 <sup>a,b</sup>	5844 <sup>a</sup>	40.66 <sup>a</sup>
$T_5$	54.32 <sup>d</sup>	518.19 <sup>c</sup>	199.55 <sup>e</sup>	3.43 <sup>c</sup>	9.23 <sup>b,c</sup>	147.89 <sup>d</sup>	9.77 <sup>d,e</sup>	10.64 <sup>c</sup>	37.70	3231 <sup>d</sup>	5368 <sup>a</sup>	37.76 <sup>a</sup>
$T_6$	76.12 <sup>a</sup>	910.7 <sup>a</sup>	370.93 <sup>a</sup>	4.94 <sup>a</sup>	17.52 <sup>a</sup>	295.67 <sup>a</sup>	14.04 <sup>a</sup>	27.82 <sup>a</sup>	40.31	4292 <sup>a</sup>	6262 <sup>a</sup>	41.30 <sup>a</sup>
$T_7$	64.21 <sup>b,c</sup>	671.6 <sup>b</sup>	316.44 <sup>b,c</sup>	3.94 <sup>b</sup>	11.13 <sup>b</sup>	265.54 <sup>a,b</sup>	11.63 <sup>b,c</sup>	18.53 <sup>b</sup>	39.61	3867 <sup>b,c</sup>	5911 <sup>a</sup>	39.52 <sup>a</sup>
$T_8$	61.15 <sup>c,d</sup>	576.5 <sup>c</sup>	282.41 <sup>c,d</sup>	3.54 <sup>b,c</sup>	8.96 <sup>c</sup>	236.43 <sup>b,c</sup>	11.23 <sup>b,c,d</sup>	17.97 <sup>b</sup>	38.09	3704 <sup>b,c</sup>	5801 <sup>a</sup>	39.12 <sup>a</sup>

See Table1 for treatment details

Individual values in column with the same letter are not significantly different at  $p < 0.05$  according to DMRT

**Table 3.** Effect of nutrient management on soil enzymatic activity, nutrient uptake and economics of wheat

Treatment	Enzymatic activity		Nutrient uptake			Economic Analysis			
	DHA ( $\mu\text{g}$ TPF/gm/24 hr)	AP ( $\mu\text{g}$ PNPP/gm/ hr)	N (kg/ha)	P (kg/ha)	K (kg/ha)	Cost of cultivation ( $\times 10^3\text{₹/ha}$ )	Gross return ( $\times 10^3\text{₹/ha}$ )	Net return ( $\times 10^3\text{₹/ha}$ )	B:C
T <sub>1</sub>	9.2 <sup>b,c</sup>	23.18 <sup>e</sup>	43.6 <sup>f</sup>	5.57 <sup>b</sup>	49.6 <sup>c</sup>	24.56 <sup>c</sup>	29.88 <sup>e</sup>	5.32 <sup>f</sup>	1.22 <sup>e</sup>
T <sub>2</sub>	11.2 <sup>a</sup>	28.45 <sup>a,b</sup>	102.2 <sup>c,d</sup>	8.02 <sup>a,b</sup>	91.4 <sup>b</sup>	31.80 <sup>a,b</sup>	70.36 <sup>a,b</sup>	38.57 <sup>b,c</sup>	2.21 <sup>b</sup>
T <sub>3</sub>	10.0 <sup>a,b,c</sup>	25.29 <sup>c,d</sup>	96.2 <sup>c,d</sup>	8.1 <sup>a,b</sup>	89.4 <sup>b</sup>	30.43 <sup>a,b</sup>	62.44 <sup>c,d</sup>	32.01 <sup>d</sup>	2.05 <sup>c</sup>
T <sub>4</sub>	11.3 <sup>a</sup>	28.69 <sup>a</sup>	108.3 <sup>b</sup>	9.3 <sup>a,b</sup>	104.3 <sup>a,b</sup>	31.80 <sup>a,b</sup>	72.21 <sup>a,b</sup>	40.42 <sup>a,b</sup>	2.27 <sup>b</sup>
T <sub>5</sub>	9.2 <sup>b,c</sup>	23.18 <sup>e</sup>	86.7 <sup>e</sup>	11.08 <sup>a,b</sup>	95.1 <sup>b</sup>	36.06 <sup>a</sup>	58.16 <sup>d</sup>	22.10 <sup>e</sup>	1.61 <sup>d</sup>
T <sub>6</sub>	11.1 <sup>a,b</sup>	27.92 <sup>b</sup>	118.6 <sup>a</sup>	16.22 <sup>a</sup>	114.1 <sup>a</sup>	32.78 <sup>a,b</sup>	77.27 <sup>a</sup>	44.49 <sup>a</sup>	2.36 <sup>a</sup>
T <sub>7</sub>	10.4 <sup>a,b</sup>	26.17 <sup>c</sup>	105.7 <sup>b,c</sup>	10.65 <sup>a,b</sup>	90.0 <sup>b</sup>	30.93 <sup>a,b</sup>	69.62 <sup>a,b,c</sup>	38.69 <sup>b,c</sup>	2.25 <sup>b</sup>
T <sub>8</sub>	10.3 <sup>a,b</sup>	25.06 <sup>c,d</sup>	101.6 <sup>c,d</sup>	9.67 <sup>a,b</sup>	99.4 <sup>a,b</sup>	32.31 <sup>a,b</sup>	66.68 <sup>b,c</sup>	34.37 <sup>c,d</sup>	2.06 <sup>c</sup>

See Table 1 for treatment details

Individual values in column with the same letter are not significantly different at  $p < 0.05$  according to DMRT

potassium through MOP also made it easy for the plant for its uptake without much loss. This findings was collaborated with the findings of Patel et al. (2022).

**Economics:** Gross return ( $\text{₹}77.27 \times 10^3/\text{ha}$ ), net return ( $\text{₹}44.49 \times 10^3/\text{ha}$ ) and benefit: cost (B:C) ratio (2.36) were higher in treatment T<sub>6</sub> followed by T<sub>4</sub> (Table 3).

### CONCLUSION

Wheat responded very well to the foliar application of nano urea along with split application of MOP. Application of split dose of fertilizer along with nano urea markedly improved the plant growth, yield attributes, and grain yield of wheat as compared to traditional practices. The basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% solution of Nano Urea at CRI and flag leaf stage, increased the grain yield to the tune of 236 kg/ha when it was supplemented with 50% RDF. It was further increased to about 584 kg/ha when an additional potash was applied as foliar spray @1% solution along with nano urea as basal dose of 50:60:20 kg NPK/ha and foliar spray with 0.5% Nano Urea and 1% MOP solution at CRI and flag leaf stage. Thus combined application of nano fertilizer as foliar spray and MOP (in split doses) could be more acceptable in the growth, yield and economics of wheat in the red and lateritic soils of West Bengal.

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Received 05 October, 2024; Accepted 17 January, 2025





# Morphological and Chemical Studies for *Pulicaria undulata* L. (Compositae) in Iraq

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**Abstract:** The morphological features of *Pulicaria undulata* (L.) C. A. Mey. were observed. This species is a perennial plant, twiggy subshrub 30-120 cm high with a deep root system, leaves are lanceolate, semi-amplexicaul and decurrent on one side, inflorescence is in irregular cymes, peduncles 4-5 cm long, involucre 5-7 × 3-4 mm, phyllaries numerous, linear, brown, hairy or glabrous. Kaempferol, kaempferol 3-methyl ether, 6-methoxykaempferol, quercetin, quercetin 3-methyl ether, quercetin 3,7-dimethyl were estimated using HPLC chromatography.

**Keywords:** Compositae, Chemical profile, Iraq, Morphological study, *Pulicaria undulata*

*Pulicaria undulata* (L.) C. A. Mey. (1831) (syn. *Francoeuria crispa* subsp. *crispa*, *F. crispa* var. *crispa*, *Pulicaria crispa* subsp. *crispa*, *P. crispa* var. *crispa*, *P. crispa* var. *gracillima* Maire, *P. undulata* var. *undulata*, belongs to the family Compositae (Asteraceae) (GBIF Secretariat 2020). This is native in Iraq, Iran, Yemen, Afghanistan, Algeria, Benin, Chad, Djibouti, Egypt, Eritrea, Ethiopia, Gulf States, India (Dawar et al., 2002). This species very important medicinal plant known as *Dethdath*, and used medicinal plants to make traditional medicines all over the world since ancient times. The branches and flowers are used to prepare powders to repel insects (Khansaa et al., 2017). This species is used for a traditional medicine which act as tonic, antispasmodic, antihypoglycemic drugs and ingredients of perfumes (Ghazanfar and Edmondson 2019) and is rich source for large number of bioactive compounds such as flavonoids which are the secondary plant metabolites and are compounds of low molecular weight. They are chemically polyphenolic and their nature offers a common structure, benzo- $\gamma$ -pyrone (Ahmed and Ibrahim 2018). This study aimed to characterize the plant through morphologic characteristics and to evaluate the flavonoids present in it.

## MATERIAL AND METHODS

Twelve plant were collected from Al-Zafaraniyah/ Baghdad- Iraq in 2020 during the flowering period in April at 3 geographical locations, the coordinate of these stations was 33°15'49.0"N 44°29'16.6"E, 33°15'44.4"N 44°29'13.1"E and 33°15'34.7"N 44°29'17.4"E. The taxonomical key was used to identify the specimens of this species for precise identification (Ghazanfar and Edmondson 2019). After being air dried, the aerial parts of this species were grounded in a

mortar and flavonoids were isolated from *Pulicaria undulata* in pure form. Five grams of plant material separated in 100 ml of methanol using maceration (48 h). Then removed in the vacuum with the temperature at 50°C and the extracts were freeze-dried (Romanik et al., 2007).

## RESULTS AND DISCUSSION

**Morphological study:** *Pulicaria undulata* is perennial plant and twiggy subshrub were 30-120 cm high with a deep root. Plant branches from the base, white-floccose or light gray in color, densely glandular, and take the form of extensive cushions. Its branches are many and may intertwine. The leaves are very long at the bottom and shorter towards the top with shape as lanceolate, semi-amplexicaul and decurrent on one side. The margin of the leaves are very clearly undulating, 2-25 × 1-5 mm, base almost auriculate (Fig. 1). Inflorescence is irregular cymes, peduncles 4-5 cm long, involucre 5-7 × 3-4 mm, phyllaries numerous, linear, brown, hairy or glabrous Ghazanfar and Edmondson (2019) also mentioned similar observation. Corolla yellow, 2.75-3 mm long, the ligule only 1 mm, and the disc florets 2.5-3.5 mm long, with glandular hairs. Cypsela 0.8 mm long, glabrous, brown, 5-ribbed, the bristles with a short narrow tuft of slightly longer hairs at the tip. Outer pappus is ring of scales and inner pappus of bristles (short stiff hair) joined at the base into a tube, each bristle with a narrow apical tuft of slightly longer hairs. The morphology gave as clear features of this species very useful for the delimitation and identification. There were two types in trichomes that spread densely in its parts. The non-glandular and glandular which spread and concentrated in all parts organs: leaves, stems and flowers (Abid and Kaiser 2002) Trichome diversity were divided into 4 groups.

**Non-glandular trichome:** These are found in all plant parts and are simple non-glandular unicellular trichomes, different lengths ranging from short to very long about 10-860 $\mu$ m, curved all with acute apex (Fig. 2. A 1- A 3) (Krak and Mraz 2008).

**Non-glandular multicellular trichomes:** These are also found in all parts of plants, some trichome fragmented from ending with a thin flagellum, others have basal cell broader, 3- 7 cells long and the apical cell like a conical shape, others consists of two cells of different lengths, straight or curved and all with obtuse apex (Fig. 2. A 3- A7) (Al-dobaissi et al., 2016).

**Stellate trichomes:** The sparse stellate hairs are on the involucral bracts (Fig. 2. A8).

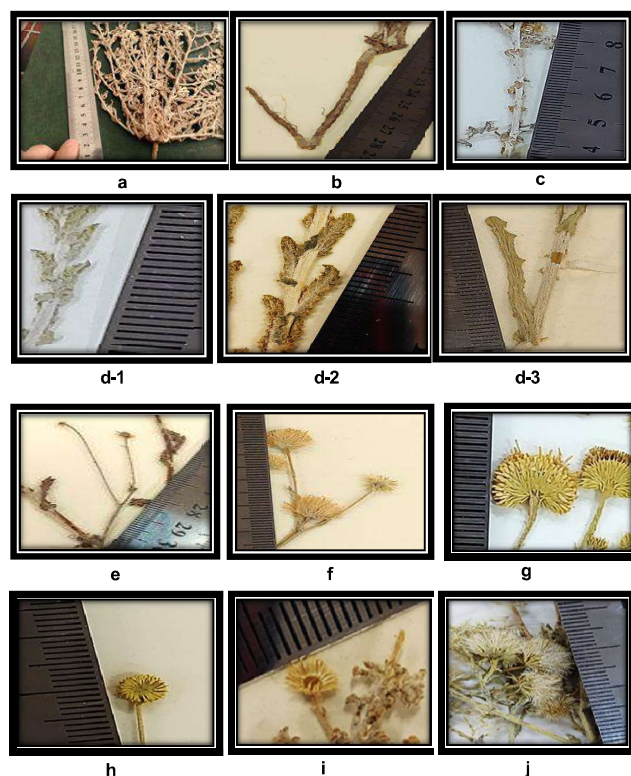
**Glandular trichome:** These were multicellular divided into head and stalk (Fig. 2. B1) (Valkama et al., 2003, Ciccarelli et al., 2007).

**Chemical profile:** Six different flavonoids were extracted and identified: Kaempferol, kaempferol 3-methyl ether, 6-methoxykaempferol, quercetin, quercetin 3-methyl ether, quercetin 3,7-dimethyl ether (Fig. 3 ). The peaks for target flavonoids eluted at retention times 47.82, 52.38, 44.71, 34.41, 83.22 and 40.32 min, respectively, through the sample extract (Table 1). Fahmi et al (2019) also made similar

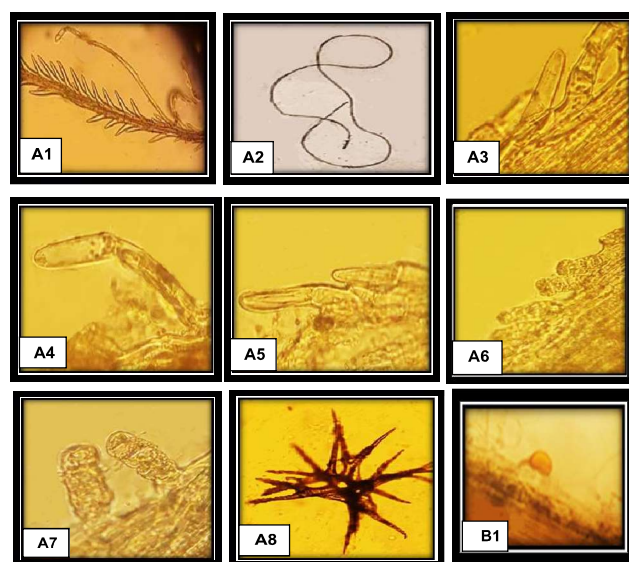
observation. Kaempferol recorded the highest value of 160.55 but quercetin recorded the lowest value both in retention time and area which was 36.66 and 34.41, respectively. The highest concentration recorded for kaempferol was 0.26 mg/mL. Flavonoids protect plants against different biotic and abiotic stresses, and responsible resistance to frost,

**Table 1.** Flavonoids component with retention time and area of this species

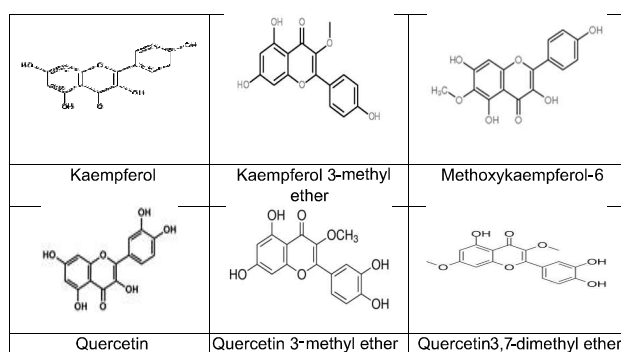
Flavonoid compounds	Retention time (mins)	Area	Concentration mg/mL
Kaempferol	47.82	160.55	0.26
Kaempferol 3-methyl ether	52.38	50.32	0.11
6-Methoxykaempferol	44.71	40.13	0.05
Quercetin	34.41	36.66	0.04
Quercetin 3-methyl ether	83.22	90.71	0.15
Quercetin 3,7-dimethyl ether	40.32	110.53	0.20



**Fig. 1.** Morphological characteristics of *Pulicaria undulata*: Whole plant, b: Root, c: the stem, d1-3: Different lengths of leaves, e-i: Inflorescence, j: Pappus calyx



**Fig. 2.** Morphological characteristics for the trichomes of the species *Pulicaria undulata* A1-8: Non-glandular trichomes (300x), B1: Glandular trichomes (400x)



**Fig. 3.** Morphological form for the flavonoid compounds of the species *P. undulata*

hardiness, drought resistance, also have a functional role for plant heat acclimation and freezing tolerance (Vieira et al., 2003). Mierziak et al. (2014) reported that the essential oil of the aerial parts for *P. gnaphalodes* has shown the presence of flavonoids, but studies on the concentrations of flavonoids are very rare in Iraq.

### CONCLUSION

The study verified the morphological features of *Pulicaria undulata* (L.) C. A. Mey. (Asteraceae) which collected from Al-Zafaraniyah/ Baghdad-Iraq. Trichomes (hairs) were mainly divided into non-glandular and glandular. Various chemical kaempferol, kaempferol 3-methyl ether, 6-methoxykaempferol, quercetin, quercetin 3-methyl ether, quercetin 3,7-dimethyl were estimated by HPLC chromatography. This will be helpful for medicinal use and further investigations.

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# Quantifying Response of Irrigation and Anti-Transpirants on Quality, Water Use Efficiency and Economics of Indian Mustard (*Brassica juncea* L.) Czern & Coss

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**Abstract:** The present investigation was carried out during 2017-18 and 2018-19 at Regional Research Station, Bawal to study the effect of irrigation regimes and anti-transpirants on quality, WUE and economics of Indian mustard. The experiment was laid out in split plot design with three irrigation regimes (control, one irrigation at 40 DAS and two irrigations at 40 and 75 DAS) and two varieties (RH-725 and RH-749) in main plots, whereas, anti-transpirants (Control, PMA @ 250 ppm, 6 % kaolin and PMA @ 250 ppm + 6% kaolin at 45 and 90 DAS) in sub plots. The significantly higher oil content and oil yield, maximum consumptive use of water (CU), water use efficiency (WUE), gross returns (GR), net returns (NR), B-C ratio as well as net income per day (NIPD) were in crop irrigated at 40 and 75 DAS. In anti-transpirants, highest oil yield, CU, WUE, gross return and net returns, B-C ratio and net income per day (NIPD) were with application of PMA @ 250 ppm + 6% kaolin at 45 and 90 DAS as compared to control but it was statistically at par with application of kaolin 6 % at 45 and 90 DAS t. Variety RH-749 performed better over to RH-725 with respect to the yield, CU, WUE and economic returns. However, varieties did not exhibit a significant influence on the seed oil and glucosinolate content.

**Keywords:** Anti-transpirants, Indian mustard, Varieties, Water use efficiency

Indian mustard is a *rabi* season crop grown in diverse agro-climatic conditions, ranging from northern hills to down south under irrigated and rainfed conditions. In global oilseeds scenario, India occupies 12-15 per cent of oilseeds area, 6-7 per cent of vegetable oils production, 9-11 per cent of the total edible oil consumption and 14 per cent of vegetable oil imports (Bhukhar et al., 2022). It is cultivated on 10 million hectares of area with 12.90 million tonnes of production and 1209 kg/ha productivity in India during 2023-24, whereas in Haryana, crop grown on 0.72 million hectares area and produce 1.40 million tonnes with average productivity of 1944 kg/ha (Anonymous 2023).

The production of mustard is not being fully exploited because of the lack of proper information of water requirement. Adequate supply of irrigation water helps in proper utilization of plant nutrients, resulting in better crop growth and higher yield. Therefore, there is need to find out some appropriate solution to fulfil the irrigation requirement of mustard crop. Application of irrigation at flowering and siliqua development stage significantly increases the CU and oil content (Shivran et al., 2018). For increasing the productivity of mustard crop the improved varieties which are capable of giving high yields need to be cultivated. Kumar et al. (2017) observed that mustard variety RH-749 registered

significantly higher yield, oil content and net returns and B-C ratio. Kumar et al. (2018) revealed that genotypes RH-725 recorded higher oil content, oil yield and net returns.

The anti-transpirants are used in agricultural field which reduce transpiration rate by reducing the size and number of stomata and gradually hardening them to stress (Kumar et al., 2018). Spraying of anti-transpirants, reduces transpiration loss of water from vegetative parts of the plant, go a long way in economizing water and making more water available to the plant for productive purpose. The combined spray of PMA @ 250 ppm + kaolin (6%) at 45 and 90 DAS recorded significantly higher oil content, net returns, B-C ratio, WUE and nutrient use efficiency (Rajput 2012) and Kumar et al. (2017). The present investigation was carried out to evaluate the Indian mustard varieties under different irrigation regimes and anti-transpirants.

## MATERIAL AND METHODS

**Study area:** The experiment was conducted at Regional Research Station, Bawal (Fig. 1) CCSHAU, Hisar (India) during *rabi* season 2017-18 and 2018-19. The site is located in south-west (SW) zone of Haryana which comes under arid and semi-arid region having latitude and longitude of 28.10N, 76.50 E, respectively above mean sea level of 266 meters.

**Weather:** The climate of the site is arid and semi-arid type, with severe cold days in winter and hot sunny days in summer season. The mean maximum temperature touches as high as 48°C during summer season. However, minimum temperature of 2-3°C is recorded during winter months. The south-west monsoon (SWM) brings rain from July-September providing 80-85 % of total annual rainfall of the region. The weather remains dry except light rainfall during the months from October-April. However, the high temperature is prevailing in June month.

**Sampling and analyses:** The experimental soil was sandy loam in texture. The composite soil sample was collected before sowing and brought to laboratory. The soil samples were air dried, grinded and passed through 2 mm sieve for the analysis of physico-chemical properties. The pH of the experimental soil was neutral in nature (8.24) having electrical conductivity of 0.19 ds/m (Rechard, 1954). The determination of organic carbon by Walkely and Black (1947), available nitrogen by Subbaiah and Asija (1956), available phosphorus by Olsen et al. (1954) and available potassium by Jackson, 1973 and it indicated that soil content 0.23 %, 148 kg/ha, 14.22 kg/ha and 208 kg/ha of organic carbon, available nitrogen, available phosphorus and available potassium, respectively.

**Experimental setup:** The experiment was laid out in split plot design having twenty-four treatment combinations with three replicates. The experiment had three irrigation regimes ( $I_1$ : control,  $I_2$ : one irrigation at 40 DAS,  $I_3$ : two irrigations at 40 and 75 DAS), and two varieties ( $V_1$ : RH-725,  $V_2$ : RH-749) in main plots, whereas, four anti-transpirants ( $A_1$ : control,  $A_2$ : PMA @ 250 ppm at 45 and 90 DAS,  $A_3$ : 6 % kaolin at 45 and 90 DAS and  $A_4$ : PMA @ 250 ppm + 6% kaolin at 45 and 90 DAS) in sub plots. Pre-sowing irrigation was applied before

seed bed preparation to ensure adequate soil moisture. Mustard variety RH-749 and RH-725 was sown at 30 cm row spacing. Application of irrigation and anti-transpirants as per the treatment. The seed and stover yield per plot were recorded and then converted into kg/ha.

#### Observation Recorded

**Oil content (%):** The oil content was determined in percentage by steam distillation method (AOAC 1995).

$$\text{Oil yield (kg/ha)} = \frac{\text{Weight of empty flask}}{\text{Weight of flask with oil}} \times 100$$

**Oil yield (kg/ha):** The seed yield obtained for each treatment was multiplied by the percent oil content in seed of respective plot for calculation of oil yield which was reported in kg/ha.

$$\text{Oil yield (kg/ha)} = \frac{\text{Oil content in seed} \times \text{Seed yield (kg/ha)}}{100}$$

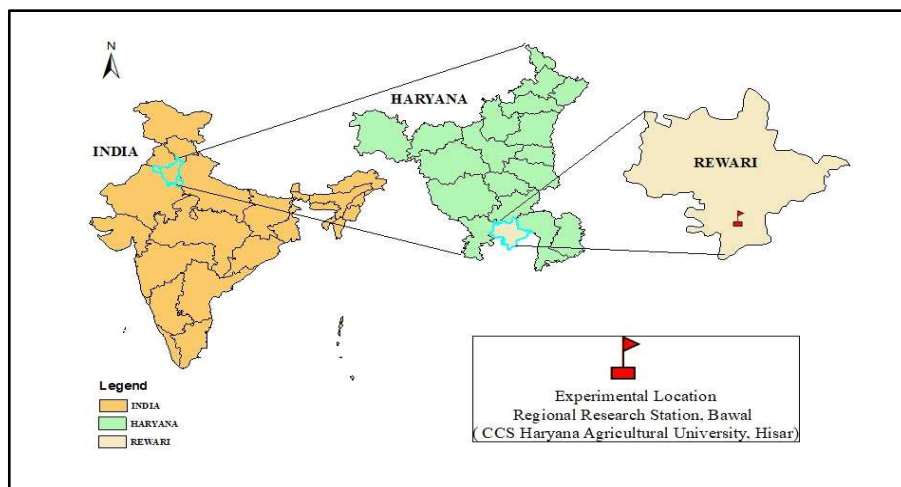
**Glucosinolate content (%):** Spectrophotometric estimation was done using methanolic extract<sup>15</sup> prepared from the same genotypes by homogenizing 0.2 g defatted seed meal in a 2 ml vial with 80 % methanol. Total glucosinolates was calculated by putting the OD of each sample at 425 nm into the predicted formula  $y = 1.40 + 118.86 \times A_{425}$  (Kumar et al., 2004).

**Moisture content (%):** Soil profile moisture content was determined by gravimetric method. Plot wise soil samples were drawn at depth intervals of 0 to 15, 15-45 and 45-90 cm soil layers before and after each irrigation. Soil moisture content of soil samples was worked out by using the following formula:

$$\text{Soil moisture content (\%)} = \frac{W_1 - W_2}{W_2} \times 100$$

Where,  $W_1$  = Fresh weight of soil (g),  $W_2$  = Dry weight of soil (g)

**Consumptive use (mm):** The CU was computed from the



**Fig. 1.** Experimental Location, RRS, Bawal, CCS Haryana Agricultural University, Hisar, Haryana

water balance (Dastane 1972).

$$CU = \sum_{i=1}^n \frac{M_{1i} - M_{2i}}{100} \times Asi \times Di$$

Where,

CU = Consumptive use of water between two successive sampling periods (cm)

$M_{1i}$  = Soil moisture at first sampling in  $i^{\text{th}}$  layer (%)

$M_{2i}$  = Soil moisture at second sampling in  $i^{\text{th}}$  layer (%)

Asi = Apparent specific gravity of the  $i^{\text{th}}$  soil layer

Di = Depth of the  $i^{\text{th}}$  layer of soil (cm)

N = Number of soil layers in the root zone

**Water use efficiency (kg/ha/mm):** The WUE was worked out with the help of the following formula:

$$WUE \text{ (kg/ha/mm)} = \frac{\text{Seed yield (kg/ha)}}{\text{consumptive water use (mm)}}$$

**Statistical analysis:** Experimental data were statistically analyzed by using SPSS software.

**Table 1.** Average mean weekly values of weather parameters during cropping season (2017-18 and 2018-19)

Parameters	Unit	2017-18	2018-19
Rainfall	(mm)	18.8	34.4
Temperature	(max.)	27.14	24.75
Temperature	(min.)	9.30	9.00
Relative humidity	M (%)	84.75	87.16
	E (%)	30.62	38.83
Bright sunshine	(hrs.)	6.62	5.90
Wind speed	(km/h)	2.22	2.78

**Table 2.** Oil content (%), oil yield (kg/ha) and glucosinolate content (%) of Indian mustard as influenced by irrigation levels, varieties and anti-transpirants (Pooled of 2017-18 and 2018-19)

Treatment	Oil content (%)	Oil yield (kg/ha)	Glucosinolate content ( $\mu\text{mol/g}$ )
<b>Irrigation levels (I)</b>			
I <sub>1</sub> - Control (No post-sowing irrigation)	38.7	543.0	79.1
I <sub>2</sub> - One irrigation at 40 DAS (At pre-bloom stage)	39.4	673.3	80.1
I <sub>3</sub> - Two irrigation at 40 and 75 DAS (At pre-bloom + pod filling stage)	39.7	788.7	82.2
CD (p=0.05)	0.6	95.9	NS
<b>Varieties (V)</b>			
V <sub>1</sub> -RH-725	39.1	626.5	79.7
V <sub>2</sub> -RH-749	39.4	710.3	81.2
CD (p=0.05)	NS	78.3	NS
<b>Anti-transpirants (A)</b>			
A <sub>1</sub> - Control	39.1	592.2	79.7
A <sub>2</sub> - PMA @ 250 ppm at 45 and 90 DAS	39.2	652.7	81.2
A <sub>3</sub> - Kaolin 6 % at 45 and 90 DAS	39.3	683.6	79.8
A <sub>4</sub> - PMA @ 250 ppm + Kaolin (6%) at 45 and 90 DAS	39.4	744.8	81.1
CD (p=0.05)	NS	71.7	NS

## RESULTS AND DISCUSSION

**Effect of irrigation:** The successive increase in number of irrigations at different phenological stages increased the oil content in both the years (Table 2). Application of two irrigations at 40 and 75 DAS established its superiority by producing significantly the highest oil yield. In general, oil yield decreased significantly with each number of irrigations at different phenological stages in both the years and was statistically at par with one irrigation at 40 DAS only during 2018-19. The different irrigation regimes did not exert their significant influence on the glucosinolate content, respectively during both years. Probably adequate supply of moisture helps in the greater uptake of nitrogen, which in turn, higher the oil content in seed. Therefore, the higher seed yield was responsible for higher oil yield under I<sub>3</sub>, whereas, increased in temperature at maturity stage under control condition, increased the loss of oil through volatilization might have reduced the oil content of seed. Contrarily, a significant response of irrigation on oil content and oil yield of mustard was Observed in earlier studies (Tyagi and Upadhyay 2017, Shivran et al., 2018, Mishra et al., 2019, Piri et al., 2019 and Kumar and Dhillon 2023).

The soil moisture content under in control declined consistently from sowing till harvest (Table 3). The decline in moisture content was faster in beginning and at crop maturity stage than during mid-season. The irrigation frequency increased the soil moisture content considerably by application of two irrigations at 40 and 75 DAS followed by one irrigation at 40 DAS. At harvest, soil profile under both

control and irrigated condition exhausted completely. Soil moisture use was maximum under 0-15 cm layer and it declined with the increased in profile depth during both years. The increase in soil moisture content might be due to timely water supply through irrigation. The moisture content at pre-bloom stage before irrigation under all the irrigation treatments remained more or less same because at this stage none of the plots were irrigated. However, at pod filling stage moisture content in all the three depths (0-15, 15-45

and 45-90 cm) were higher under I<sub>3</sub> treatment because at this stage crop had received two irrigations at pre-bloom and pod filling stage, whereas, under I<sub>2</sub> treatment received only one irrigation at pre-bloom stage.

The increase in consumptive water use and water use efficiency by application of two irrigations at 40 and 75 DAS was 30.3 per cent and one irrigation at 40 DAS was 14.8 per cent over control (Table 4). Consumptive use of water by the crop increased progressively and appreciably with increase

**Table 3.** Soil moisture content (%) at varying depth of Indian mustard as influenced by irrigation levels, varieties and anti-transpirants (Pooled of 2017-18 and 2018-19)

Treatments	Initial	1 <sup>st</sup> Irrigation						2 <sup>nd</sup> Irrigation						Harvest		
		Before irrigation			After irrigation			Before irrigation			After irrigation					
		0-15 cm	15-45 cm	45-90 cm	0-15 cm	15-45 cm	45-90 cm	0-15 cm	15-45 cm	45-90 cm	0-15 cm	15-45 cm	45-90 cm	0-15 cm	15-45 cm	45-90 cm
Irrigation levels (I)																
I <sub>1</sub>	13.4	7.0	8.4	10.4	6.8	8.1	10.1	5.0	6.1	7.9	4.8	5.8	7.6	3.0	3.8	5.5
I <sub>2</sub>	12.7	7.2	8.6	10.5	10.7	12.4	14.9	9.5	10.4	12.0	6.8	8.0	9.7	5.9	6.1	6.5
I <sub>3</sub>	12.5	7.1	8.0	10.4	10.9	11.3	13.6	9.0	11.9	12.2	11.6	13.4	16.0	7.7	8.6	10.7
Varieties (V)																
V <sub>1</sub>	12.7	7.1	8.3	10.0	9.4	10.5	12.7	7.8	9.6	10.8	7.6	9.1	11.1	5.6	6.3	7.5
V <sub>2</sub>	13.1	7.1	8.4	10.9	9.5	10.7	13.0	7.8	9.4	10.6	7.9	9.0	11.1	5.5	6.0	7.6
Anti-transpirants (A)																
A <sub>1</sub>	12.8	7.1	8.7	10.3	9.6	10.5	12.5	8.0	9.7	10.8	7.7	9.3	11.2	5.6	6.4	7.5
A <sub>2</sub>	12.4	7.2	8.7	10.2	9.5	11.2	12.9	7.9	9.6	10.3	7.9	9.4	11.1	5.5	6.2	7.7
A <sub>3</sub>	13.1	6.9	7.9	10.5	9.2	10.2	13.3	7.8	9.2	11.2	7.5	8.6	11.0	5.4	5.9	7.4
A <sub>4</sub>	13.3	7.3	8.0	10.7	9.6	10.6	12.8	7.7	9.3	10.5	7.8	9.0	11.1	5.6	6.0	7.7

See Table 2 for treatment details

**Table 4.** Consumptive use of water (mm), water use efficiency (kg/ha/mm) and economics of Indian mustard as influenced by irrigation levels, varieties and anti-transpirants (Pooled of 2017-18 and 2018-19)

Treatment	Consumptive use of water (cm)			Consumptive use of water (mm)	Water use efficiency (kg/ha/mm)	Gross returns (₹/ha)	Net returns (₹/ha)	Net income per day (₹/ha/day)	B: C ratio
	0-15	15-45	45-90						
Irrigation levels (I)									
I <sub>1</sub>	6.9	6.3	5.1	180.7	7.8	67671	25557	184	1.6
I <sub>2</sub>	8.1	7.1	5.7	207.3	8.3	82923	39609	281	2.0
I <sub>3</sub>	9.5	7.8	6.3	235.3	8.4	95452	50938	357	2.2
Varieties (V)									
V <sub>1</sub>	8.1	7.0	5.6	205.5	7.8	77574	34260	246	1.8
V <sub>2</sub>	8.3	7.1	5.7	210.1	8.6	86456	43142	301	2.0
Anti-transpirants (A)									
A <sub>1</sub>	8.2	6.9	5.6	205.0	7.4	73616	31508	226	1.8
A <sub>2</sub>	8.1	7.1	5.6	206.8	8.1	80420	36728	261	1.9
A <sub>3</sub>	8.1	6.9	5.8	207.2	8.4	83711	40775	287	2.0
A <sub>4</sub>	8.3	7.2	5.7	212.2	8.9	90315	45795	322	2.0

See Table 2 for treatment details

in number of irrigations because irrigation increased the available water in the soil profile and this facilitated more loss of water through evapotranspiration as compared to no irrigation. Frequent irrigations though, necessary for yield maximization usually lower the water use efficiency because moist or wet surface of soils results in increased loss of soil moisture through evaporation but simultaneously increases consumptive use. These findings are in close proximity with Tyagi and Upadhyay (2017) and Shivran et al. (2018).

Significantly maximum gross monetary return, net realization, net income per day and benefit cost ratio was with the application of two irrigations at 40 and 75 DAS followed by one irrigation at 40 DAS (Table 4). The mean increase in net realization due to application of two irrigations at 40 and 75 DAS was to the extent of 28.8 per cent over  $I_2$  and 99.2 per cent over  $I_1$ . The increase in seed yield under this treatment had appreciably been compensated by the increased expenses on account of increase in irrigation and hence, resulted in greater net return, net income per day and benefit cost ratio. These results are in consonance with Verma et al. (2014) and Tyagi and Upadhyay (2017).

**Effect of variety:** The significantly the highest oil yield was recorded under RH-749 over RH-725, respectively during both years. Percentage increase in oil yield with RH-749 were 13.3 per cent over RH-725. Whereas, varieties did not exhibit their significant influence on the oil content of seed and glucosinolate content (Table 2).

An appraisal data in Table 3 indicated that soil moisture content among genotypes there was very little difference in moisture extraction from different layers during both years. Whereas, among the different genotypes there was very little difference in moisture extraction from different layers, Consumptive use and water use of efficiency (Table 4) was recorded significantly higher with RH-749. Percentage increase in WUE with RH-749 were 10.3 and 11.0 per cent over RH-725. The data reveals in Table 4 that mustard variety RH-749 accrued the maximum gross and net realization, net income per day and B:C ratio. Percentage increase in net realization with RH-749 was 25.9 per cent over RH-725. Genetic potential of a particular variety plays a role in exploitation of higher yield and there by remuneration. Results are in concurrence with those of reported earlier by Kumar et al. (2017), Singh et al. (2017), Meena et al. (2017), Kumar et al. (2018), Yadav et al. (2018), Jaiswal et al. (2019), Rajyalakshmi et al. (2019), Maurya et al. (2022) and Tamboli et al. (2024).

**Effect of anti-transpirants:** The experimental results indicated that effect of different anti-transpirants was noted on oil yield but did not exhibit any influence on oil content and glucosinolate content (Table 2). As compared to control,

application of PMA @ 250 ppm + kaolin (6%) at 45 and 90 DAS recorded significantly higher oil yield and was at par with both application of kaolin 6 % at 45 and 90 DAS only during 2018-19. The above treatment directly influenced the physiology of the crop and reduced the losses of moisture from field and increased moisture utilization and uptake of nutrients, which ultimately resulted in higher grain yield of mustard and its quality. The oil yield is dependent on oil content in seed and seed yield. Therefore, the higher seed yield was responsible for higher oil yield under application of PMA @ 250 ppm + kaolin (6%) at 45 and 90 DAS during both years. The results are also close agreement with those reported by Rajput (2012), Badukale et al. (2015), Kumar et al. (2017), Kumar et al. (2018) and Mphande et al. (2020).

The consumptive use of water and water use efficiency (Table 4) of mustard increased significantly due to application of PMA @ 250 ppm + kaolin (6%) at 45 and 90 DAS followed by application of kaolin 6 % at 45 and 90 DAS. The above treatment directly influenced the physiology of the crop and reduced the losses of moisture from field and increased moisture utilization. The application of PMA and kaolin alone and in combination through foliar spray may be an option to improve the biometric parameters by reducing the size and number of stomata and gradually hardening them to stress. Thus, it can be helpful in economizing water and making it available to the plant for growth and seed production. The present findings are agreement with Rajput (2012), Badukale et al. (2015), Kumar et al. (2017), Kumar et al. (2018) and Tamboli et al. (2024).

The highest gross and net monetary return, net income per day and benefit cost ratio (Table 4) accrued with application of PMA @ 250 ppm + kaolin (6%) at 45 and 90 DAS followed by application of kaolin 6 % at 45 and 90 DAS and PMA @ 250 ppm at 45 and 90 DAS over control. The mean increase in gross realization due to application of PMA @ 250 ppm + kaolin (6%) at 45 and 90 DAS was to the extent of 45.3 per cent over control. The above treatment directly influenced the physiology of the crop and reduced the losses of moisture from field and increased moisture utilization and uptake of nutrients, which ultimately resulted in higher grain yield of mustard, which might be responsible for higher net monetary return, net income per day and benefit cost ratio, which showed significant positive correlation with seed yield. These findings are in conformity with results reported by the present findings are agreement with Rajput (2012), Badukale et al. (2015), Kumar et al. (2017), Kumar et al. (2018) and Tamboli et al. (2024). Interaction effect on oil yield between levels of irrigation and anti-transpirants was significant (Table 5). The significantly higher oil yield was recorded when application of two irrigations at 40 and 75 DAS with PMA @

Add .



**Table 5.** Interaction between levels of irrigation and anti-transpirants on oil yield (kg/ha) of Indian mustard

Treatment		Oil yield (kg/ha)									
		2017-18					2018-19				
		Anti-transpirants (A)									
		A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	Mean	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	Mean
Irrigation levels (I)	I <sub>1</sub>	434.8	607.7	565.5	546.2	538.5	474.0	645.4	534.5	536.0	547.5
	I <sub>2</sub>	609.7	578.2	656.5	740.9	646.3	673.8	615.2	737.1	774.7	700.2
	I <sub>3</sub>	731.6	710.1	708.9	938.1	772.1	629.5	759.4	899.3	933.2	805.3
	Mean	592.0	632.0	643.6	741.7		592.4	673.4	723.6	747.9	
CD (p=0.05)		115.5					132.7				

See Table 2 for treatment details

250 ppm + kaolin (6%) at 45 and 90 DAS, respectively during both year and was statistically at par with treatment combinations of I<sub>3</sub>A<sub>3</sub> only during 2018-19.

### CONCLUSIONS

The higher oil content, oil yield, glucosinolate content, soil moisture content, consumptive use of water, water use efficiency, net return and B: C ratio could be obtained when mustard is grown with two irrigations, each at 40 and 75 DAS. The variety RH-749 proved to be better as compared to RH-725 in terms of oil yield, net return and consumptive use of water and water use efficiency. The application of PMA @ 250 ppm + kaolin (6%) at 45 and 90 DAS recorded significantly higher oil yield, soil moisture content, consumptive use of water and water use efficiency. Thus, higher quality, net return and consumptive use of water and water use efficiency can be achieved by sowing the mustard variety RH-749 along with application of two irrigations at 40 and 75 DAS and PMA @ 250 ppm + kaolin (6%) at 45 and 90 DAS on loamy sand soils.

### AUTHOR'S CONTRIBUTION

Dr. Y.A. Tamboli: Conducted experiment, recorded data, data analysis; Dr. J.S. Yadav: Monitoring of experiment, formulation of experiment, correction, providing assistance and data analysis; Dr. Parveen Kumar: Guidance while conducting experiment, manuscript corrections; Dr. Ajeet Singh and Kapil Malik: Data analysis and article verification.

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# Flood's Unwavering Touch: Unveiling the Socio-Economic Symphony of Ghatal Sub-division, Paschim Medinipur, West Bengal

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**Abstract:** The socio-economic challenges faced by Ghatal, West Bengal, due to frequent floods in the lower Shilavati River basin are the focus of this study. Employing a mixed-methods approach, it combines comprehensive household surveys with advanced statistical analyses to assess the impacts on the local community, particularly within the Ghatal Sub-division. The research highlights how flooding during the monsoon season exacerbates issues in low-lying areas, significantly affecting agriculture and disrupting livelihoods. Key findings reveal that 78% of households remain in flood-prone areas despite the risks, and there is a significant loss of residential and agricultural lands, posing major threats to riverbank communities. The study also emphasizes the public health risks associated with flooding, including outbreaks of waterborne diseases and reduced access to healthcare due to damaged infrastructure. By detailing the adaptive measures that communities have ingeniously adopted in response to these persistent environmental challenges, the research offers valuable insights into community resilience. Furthermore, it provides recommendations for policymakers and researchers aimed at fostering sustainable development and enhancing the capacity to address climatic challenges.

**Keywords:** Agriculture territory, Lower reaches, Sustainable development, Shilavati River Basin, Socio economic landscape

Geographically and historically, West Bengal is one of India's most prosperous states. Known for its rich ethnic and religious cultural heritage, West Bengal is also distinguished by its diverse physiographic, climatic, and environmental attributes, earning it the reputation as one of the most resourceful states in India (Adhikary 2022). A large part of the state of West Bengal is exposed to frequent natural catastrophes like floods, cyclones, droughts and landslides that often turn into disasters causing significant disruption of socioeconomic life of communities and loss of human lives and properties (Das et al., 2011). The situation in India is not better since 55 per cent of India's landmass is prone to earthquakes; 68 per cent is vulnerable to drought; 12 per cent to floods; and 8 per cent to cyclones apart from the heat waves, and severe storms (Chawla 2012). From 2000 to 2019, floods affected more than 1.5 billion people worldwide. They accounted for 44% of all global disasters during this period. This highlights the significant impact of floods on global populations (UN Office for Disaster Risk Reduction 2020; Yu et al., 2022). Floods displace millions annually, caused by heavy rain leading to river overflow. They cause secondary effects including structural damage, contamination, and disturbance of life and property, in addition to direct hazards like water contact. (Venkatesan and Mahindrakar 2020). Flooding during the monsoon season in India, especially in July and August, is driven by

unpredictable weather and intense rainfall (Chaudhury 2021). In India, the monsoon period sees various types of floods, including riverine floods, dam break floods, and storm surge floods. Flooding is a common phenomenon, occurring every one to two years. Many rural and urban areas regularly experience floods due to the variability of monsoonal behavior. The study employs rural participatory research approaches in developing four vulnerability categories namely socio-economic, ecological, engineering and political; which were used to develop indicators that aided the calculation of total community vulnerability index for each community (Antwi et al., 2015). Floods, among other natural forces like earthquakes and storms, have significantly shaped Earth's landscape. When floods interact with human settlements and infrastructure, they can cause devastating disasters (Chaudhary and Piracha 2021). In the relentless dance between water and human civilization, floods emerge as formidable specters, casting their pervasive shadows over communities and leaving indelible imprints on landscapes. Natural and human-made disasters are prevalent globally and persist without prior notice and are believed to be escalating in terms of their scale, intricacy, occurrence rate, and economic ramifications. These natural calamities, born of water overflow, transcend mere meteorological events, posing existential threats to agriculture, infrastructure, and the very fabric of human settlements. Whether provoked by

the relentless deluge of rainfall or the gradual release of snowmelt, floods unfold as harbingers of chaos, precipitating widespread damage and irrevocable change. The symphony of destruction orchestrated by floods is not confined to physical upheaval alone; it extends its reach to the displacement of entire populations, compromising water quality, and demanding urgent mitigation measures. Navigating the treacherous waters of floods requires a multifaceted approach, incorporating advanced early warning systems, the fortification of resilient infrastructure, and the implementation of sustainable water management practices. Yet, at the heart of this complex equation lies the linchpin of community preparedness—a proactive stance that becomes instrumental in minimizing the devastating impact of these natural cataclysms. In the intricate tapestry of India's climatic embrace, the monsoons unleash torrential downpours that swell rivers and water bodies to their zenith, setting the stage for the perennial drama of floods. The 42.55% of the topography stands susceptible to the capricious whims of flooding in Ghatal sub-division (Annual Flood Report 2019). The Ghatal-Daspur, in Paschim Medinipur District, the landscape epitomizes heightened susceptibility to flooding. This study explores the historical flooding patterns in Ghatal Block by assessing the socio-economic impacts on livelihoods, housing, and agriculture, and delving into adaptive strategies alongside residents' perceptions of risk and vulnerability.

## MATERIAL AND METHODS

**Study area:** The study area is nestled between coordinates N 22°31'53"N to N 22°49'26" and E 87°30'47" to E 87°48'16"E, Ghatal sub-division spans 953.09 sq.km with total cropped area 69787 hectares. Positioned in West Bengal's Paschim Medinipur district, the area boasts a unique river pattern, dividing it into three sections. Ghatal, the focal point, lies at the lowest elevation, intricately intersected by rivers like Shilabati and Rupnarayan. Excessive rainfall poses a flood threat (Dandapat and Panda 2017). Ghatal sub-division encompasses five blocks, with portraying the captivating study area and its vulnerability to natural calamities (Table 1). The survey was conducted within the research area using anthropogenic inquiry methods to engage the local community. Collaborative flood mapping efforts were complemented by symbiotic mapping ventures, and hydrological data were extracted from governmental repositories. Integration of advanced technologies like the Digital Elevation Model (DEM) and Landsat satellite imagery enhanced analytical capabilities, seamlessly blending community perspectives, governmental datasets, and cutting-edge technology within the study.

**Methodology:** This investigation systematically explores the factors contributing to flooding in the Ghatal block and the significant impacts on local livelihoods. The work combines both primary and secondary data sources to ensure a comprehensive analysis. Flood experiences, impacts, and damages were assessed through focus group discussions and key informant interviews, particularly with elderly residents and those highly exposed to river pollution is an enormous problem in developing countries like India. The main cause attribute could be lack of proper planning and ignorance of local and common people (Roy et al., 2021). Primary data is collected through interviews with local residents and administrative officials, and collaborative flood mapping efforts to capture the direct experiences of those affected. Secondary data was sourced from governmental records, including gauge data and rainfall statistics from the

**Table 1.** Study area

Area name	Block	Latitude	Longitude
Ramjibanpur	Chandrokona -1	N 22°49'26"	E 87°35'55"
Jara	Chandrokona -1	N 22°46'7"	E 87°35'2"
Kirpai Agi Farm	Chandrokona -1	N 22°43'15"	E 87°36'30"
Mohanpur	Chandrokona -1	N 22°36'33"	E 87°35'18"
Mangrul	Chandrokona -1	N 22°46'41"	E 87°42'4"
Lalghar	Chandrokona -2	N 22°43'56"	E 87°28'32"
Metela	Chandrokona -2	N 22°48'25"	E 87°30'09"
Piyardanga	Chandrokona -2	N 22°46'18"	E 87°31'51"
Ramganja	Chandrokona -2	N 22°43'50"	E 87°32'09"
Pinglash	Chandrokona -2	N 22°39'31"	E 87°31'01"
Chandrokona-	Chandrokona -2	N 22°44'04"	E 87°30'47"
Kharar	Ghatal	N 22°42'55"	E 87°40'07"
Irpala	Ghatal	N 22°44'25"	E 87°42'50"
Ajabnagar	Ghatal	N 22°39'55"	E 87°42'04"
Mansuka	Ghatal	N 22°42'43"	E 87°44'07"
Ghatal	Ghatal	N 22°39'57"	E 87°44'08"
Palaspai	Daspur -2	N 22°30'54"	E 87°46'53"
Goura	Daspur -2	N 22°31'53"	E 87°44'17"
Panchberia	Daspur -2	N 22°32'08"	E 87°44'46"
Basudevpur	Daspur -2	N 22°34'00"	E 87°43'25"
Gochati	Daspur -2	N 22°32'39"	E 87°46'04"
Chaipat	Daspur -2	N 22°34'17"	E 87°48'16"
Rajnagar	Daspur -1	N 22°35'34"	E 87°41'09"
Lankagar Hat	Daspur -1	N 22°33'27"	E 87°37'37"
Harirajpur	Daspur -1	N 22°33'48"	E 87°35'24"
Daspur	Daspur -1	N 22°35'15"	E 87°43'03"
Khanjapur	Daspur -1	N 22°38'31"	E 87°46'01"

Source: Google

sub-divisional irrigation department, to provide empirical support. Yearly damage data was compiled from reports on storm damage and losses. This was followed by flood risk assessments and the creation of flood risk maps, (Asare-Kyei et al., 2015). The study employs a range of methods for detailed analysis include immersive oral interviews with key stakeholders offer nuanced insights into flood dynamics historical flood data, tidal characteristics, and demographic information are drawn from administrative archives and scholarly literature. Geospatial analysis uses a Digital Elevation Model (DEM) and multi-temporal satellite imagery, including Landsat satellite data, to map regional slopes and floodwater paths. The Global Positioning System (GPS) is used to accurately identify flood-prone areas and inundation sites. This combination of methods provides a robust foundation for understanding and addressing the flooding issues in Ghatal.

## RESULTS AND DISCUSSION

### Climate of the explored area & its relation to flooding:

The tropical monsoon climate characterized by hot summers and evenly distributed rainfall and experiences four distinct seasons: a cool winter (mid-December to February), a warm summer (March to May), the South-West monsoon (June to

September), and a mild post-monsoon phase (October to mid-November). Flooding is a significant concern during the monsoon season, severely impacting the region (Sahoo and Sivaramakrishnan 2014). The historical flood trends (1959-2021) emphasize the extensive damage incurred with flood history (Table 2). The vulnerability of Ghatal to disasters and water contamination is increasingly exacerbated by climate change and rising pollution levels in the Bay of Bengal as observed by (Dolui and Ghosh 2013). This underscores the urgent necessity for comprehensive strategies to tackle these intertwined challenges and strengthen the resilience of local communities. In these economically challenged areas, homes are built from readily available materials despite frequent flooding. In Ghatal subdivision, residents primarily engage in animal husbandry and lumbering. The analyzed data of river water levels from highlights hydrological conditions and flood risks, with a focus on rainfall distribution (2011-2023) for the Shilabati, Rupnarayan and Old Cossy Rivers (Table 3).

### Cropping intensity in Ghatal sub-division and temporal shift due to flood:

Climate change and flood have a great impact on local people's lives including agricultural production, food security and rural live hoods (Table 5, 7). Food grain and commercial grain farming are common, with

**Table 2.** Flood chronology in Ghatal Sub division: Tabular overview (1959-2023)

Year	More than one month		Less than one month		Total loss (lakh)
	Stagnant flood water (Areasq.km)	Stagnant flood water depth (meter)	Stagnant flood water (Area sq.km)	Stagnant flood water depth (meter)	
1959	100	2	184	1.5	-
1967	100	2.5	69.3	1.5	-
1968	350	2.5	307.64	2	-
1973	208	3	150	2	247.64
1974	61	3	102.83	2	191.85
1975	104	2.5	110	2	309.5
1976	55.17	2.5	108	2	52.79
1977	100	3.5	130	2	1361.92
1978	710	3.5	356	2	5174.19
1999	78.39	3	100	2	8585.34
2000	80	3	120.85	2	16313.09
2007	232.5	3	400	2	49923.05
2013	200	3	125	2	4515.7
2015	700	3.5	330	2	10000
2017	800	3.5	425	2	13847.12
2019	400	2.6	176	2	832.51
2021	400	3.7	512	2.4	14879
2023	86	1.8	205	2	421

**Source:** Data collected from Irrigation Department, Ghatal sub division

poultry farming being significant in Chandrakona-1 and Chandrakona-2 blocks, but less so in Daspur-1. The area also hosts small-scale industries related to rice, wheat, jute, oilseeds, potatoes, pulses, and various processed foods. Showcasing variations in crop damage underscores the dynamic nature of agricultural outcomes across regions and years. The farmers of this area are mainly affected due to flood mainly *Kharif* paddy crop is totally damaged. *Kharif* vegetables are also damaged. Due to floods from July to October farmer cannot prepare proper seeds for winter cultivation. Monsoon flooding forces Ghatal farmers to rely on other crops, requiring irrigation for cultivation. Approximately 47% use River Lift irrigation, 24% depend on mini shallow tubewells, while others use deep tubewells, canals, or ponds. Agriculture during the non-monsoon period is fully reliant on these irrigation practices, which remain effective despite recurring flood risks. Majority of farmer about 60percent people generally buy the seeds from open market at higher price. About 75% agricultural lands are located in low lying areas where water logging condition prevails about 3 months (Table 2, 3).

#### Self-perceived vulnerability to flood by location and

**habitant:** The 78% of households remain in flood-prone areas, undeterred by the evident risks. Floods affect around 42% of total land area (Table 5). Between 1960 and 2000, 11% of the state's land was submerged by 13 high-magnitude and 10 medium-magnitude floods (Gayen et al., 2022). In flood days, people have been living in relief camps or on the roadside. Most have sought temporary shelter in the homes of relatives or roadside camps with their children and elderly family members while their own homes are being repaired.

**Table 4.** Affected and shifted population during flood and sources of drinkable water available during flood

Year	Flood impact: Shifted & affected population (%)	Water source (%)
2017	39.36	25
2019	1.97	28
2020	4.38	28
2021	36.41	24
2023	28.32	21

**Source:** Sub division disaster management and health department of Ghatal sub division

**Table 3.** Analysis of river depth gauge and rainfall data (2011 to2023)

Shilabati		Rupnarayan			Old Cossye	Yearly rainfall data	Flooded period in year
Banka	Gadghat	Bandar	Ranichak	Gopiganja	Kolmijore		
Highest flood Record							
P.D.L-4.7 (m)	P.D.L-8.38 (m)	P.D.L-6.24 (m)	P.D.L-4.72 (m)	P.D.L-4.2 (m)	P.D.L-.68 (m)		
D.L.-5.08 (m)	D.L.- 8.99 (m)	D.L.-6.85 (m)	D.L.-5.33 (m)	D.L.-5.03 (m)	D.L.-9.29 (m)		
E.D.L-6.9 (m)	E.D.L-9.60 (m)	E.D.L-7.46 (m)	E.D.L-5.94 (m)	E.D.L-5.64 (m)	E.D.L-9.90 (m)		
H.F.L-3.4 (m)	H.F.L-11.12 (m)	H.F.L-9.47 (m)	H.F.L-9.14 (m)	H.F.L-8.41 (m)	H.F.L-11.12 (m)		
15.69 (m)	8.47 (m)	6.95 (m)	5.91 (m)	4.42 (m)	7.95 (m)	2049.30 mm	18.06.2011 to 27.06.2011
15.34 (m)	7.56 (m)	5.39 (m)	4.78 (m)	3.22 (m)	8.11 (m)	1539.90 mm	07.09.2012 to 26.09.2012
15.58 (m)	8.53 (m)	6.70 (m)	6.06 (m)	4.57 (m)	9.76 (m)	2187.80 mm	28.07.2013 to 21.08.2013
12.14 (m)	5.09 (m)	3.59 (m)	3.81(m)	2.68 (m)	6.40 (m)	1251.60 mm	05.09.2014 to 18.09.2014
15.79 (m)	9.90 (m)	8.41 (m)	7.58 (m)	5.82 (m)	10.24 (m)	1993.80 mm	27.07.2015 to 04.08.2015
15.52 (m)	8.59 (m)	7.40 (m)	6.39 (m)	4.75 (m)	9.600 (m)	1879.20 mm	22.08.2016 to 09.09.2016
15.50 (m)	10.02 (m)	8.38 (m)	7.37 (m)	5.79 (m)	10.700 (m)	1837.40 mm	21.07.2017 to 25.07.2017
14.88 (m)	7.34 (m)	5.45 (m)	4.99 (m)	3.78 (m)	10.150 (m)	1287.40 mm	08.08.18 to 17.08.2018
12.93 (m)	6.79 (m)	5.80 (m)	4.81 (m)	2.59 (m)	7.550 (m)	1728.30 mm	27.10.2019 to 11.11.2019
14.94 (m)	7.92 (m)	6.27 (m)	6.10 (m)	4.11 (m)	9.575 (m)	1910.20 mm	22.08.2020 to 27.08.2020
16.37 (m)	10.42 (m)	8.62 (m)	7.80 (m)	5.99(m)	10.500(m)	2692.00 mm	27.07.2021 to 21.08.2021

**Source:** Irrigation Department, Ghatal sub division. (P.D.L- Primary danger level, D.L- Danger level, E.D.L-Extreme danger level, H.D.L-Highest flood level)

**Comprehensive health issues due to prolonged flooding:**

During floods, water becomes polluted, leading to waterborne diseases. Prolonged flooding in the region causes significant health issues, with many residents affected by diarrhea and cholera. Waterborne diseases, food poisoning, and snake bites are also common. In the past, the lack of medical access led to flood-related fatalities. They experience waterborne diseases such as fever, dysentery, cholera, and snake bites, as well as psychological trauma. The flood impact of Ghatal sub-division (2017-2021) on disease patterns and roads can lead to increased waterborne and vector-borne illnesses, disrupted healthcare access, and hampered emergency

response efforts, posing significant challenges to public health and community resilience shows a poetic dance between health and infrastructure challenges, reverberates with the imperative to address both facets for the fortification of community resilience shows in (Table 6). Human activities, including engineering structures and dam construction, greatly affect the river basin, altering river flow and increasing flood risks. Nearly every household in Ghatal owns a small boat (Dingi or Donga) for mobility during such times. Medical care in Ghatal block is provided by hospitals, health centers, allopathic doctors, homeopathic practitioners, and unlicensed quacks. The disruptions in local markets compound these

**Table 5.** Crop damage mouza wise of Ghatal sub-division over seven years of intense rainfall and flooding

Year	Total no. of Mouza affected in different block				
	Ghatal	Chandrakona-1	Chandrakona-2	Daspur-1	Daspur-2
2013	130	46	100	156	87
2015	130	46	100	156	87
2016	151	73	86	Nil	87
2017	153	120	120	120	120
2019	156	148	140	162	87
2020	117	30	8	71	22
2021	156	60	103	162	87
2022	121	41	25	69	59
2023	126	38	21	87	76

Source: Disaster Management Department, Ghatal sub division and personal interviews

**Table 6.** Impact of floods on medical concerns and road infrastructure (2017-2021)

Year	Medical concern (number of person)			Impaired road (km)
	Diarrhoea & Cholera	Snake bite	Other water bourn diseases	
2017	1257	32cases/Death-2	3596	99.4
2019	258	0	456	184
2020	302	1 cases /Death-1	658	152
2021	1042	21 cases	3456	142

Source: Sub division disaster management and health department of Ghatal sub division

**Table 7.** Crop damaged in Ghatal sub-division due to heavy rainfall and flood (hectare)

Sub-division total (in ha.)	Kharif/Aman paddy seed bed (hector)		Kharif/aman paddy transplanting (hector)		Vegetables (hector)		Jute (hector)		Potato/groundnut (hector)		Flower (in hector)	
	Coverage	Damaged	Coverage	Damaged	Coverage	Damaged	Coverage	Damaged	Coverage	Damaged	Coverage	Damaged
2013	6302	1383.8	22385	15211	2855	2017	600	84.5				
2015	6302	1383.8	22385	15211	2855	2017	600	84.5				
2016	59010	12909			3200	606	795	85.7				
2017	5008	3640	15775	12575	2695	2290	795	590				
2019			59465	45920	865	780			150	5	35	25
2020			58246	13180	2391	1146	720	90	57	20		
2021	3761	2526	22364	20083	2130	1855	650	390				

Source: Sub-division agriculture department and personal interviews

challenges, casting ripple effects on education and livelihoods. A discerning scrutiny of road lengths in Ghatal for 2017-2021, especially within non-pucca road categories, suggests potential deterioration, further expounded upon in our study, "Epidemiological Analysis (2017-2021): Disease Patterns and Flood-Induced Road Impact." Floods, formidable challenges that they are, cast their pervasive shadows on education and livelihoods. Stakeholder opinions converge on Ghatal as a region teetering on vulnerability, beckoning the imperative for comprehensive solutions. This review discussion advocates for a holistic approach, seamlessly integrating hydrological, geomorphological, and community-driven strategies, thus underscoring the exigency for further scholarly exploration in this pivotal domain.

### CONCLUSION

The Shilabati River experiences regular flooding due to factors like irrigation, urban development, and changes in hydrology caused by the construction of the Shilabati dam. This has resulted in significant damage to both floodplain residents and agricultural areas, with floods becoming more frequent and severe during heavy rainfall, while the river often dries up in the non-monsoon season. Urbanization has further altered the river's morphology, increasing flood risks. Existing mitigation efforts, such as the Ghatal master plan, have had limited success. Key solutions include building flood shelters, elevating infrastructure, and constructing detention storage facilities to manage floodwaters. Community involvement and sustainable water management are essential for creating long-term flood resilience in the region, making urgent action necessary to address these challenges effectively. The recurrent floods in the GhatalShilabati River severely impact low-lying areas in Paschim Medinipur, damaging homes, infrastructure, and livelihoods, particularly in agriculture, where paddy cultivation has suffered significant losses. Despite the risks, 78% of households remain in flood-prone areas, relying on vulnerable livelihoods such as animal husbandry and small-scale farming. Floods also lead to outbreaks of waterborne diseases, worsened by damaged roads and disrupted healthcare access, highlighting the need for mobile medical camps and infrastructure improvements. Human activities like dam construction further increase flood risks by altering river flow patterns. This study emphasizes the importance of holistic flood management strategies that integrate hydrological, geomorphological, and community-based solutions to enhance resilience in the Ghatal region.

### ACKNOWLEDGMENT

The authors express sincere gratitude to the Irrigation

and Waterways Department, Ghatal block, and the Record Section of the District Collectorate Office, as well as the Block Development Office of Ghatal. Special thanks are extended to the Sub-Division Block, Municipality, and the Disaster Management Department.

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# PR-proteins Mediated Disease Resistance in Cotton Genotypes against Cotton Leaf Curl Disease Upon Pretreatment with Salicylic Acid and $\beta$ -aminobutyric Acid

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**Abstract:** Cotton leaf curl disease, caused by the *Geminivirus* and transmitted by whitefly, is a major concern for cotton growers. This study observed the effect of resistance-inducing chemicals (RICs); salicylic acid (SA) and  $\beta$ -aminobutyric acid (BABA), on pathogen-related proteins, total soluble protein, disease incidence, and disease index. Both elicitors were applied @ 250  $\mu$ M using two methods: seed priming (once) and foliar spray (thrice at 5, 19, and 33 days after germination) on three cotton cultivars showing differential responses: F1378 (susceptible), LH2076 (moderately resistant), and FDK124 (resistant). The activities of proteins such as chitinase (PR-3),  $\beta$ -1,3-glucanase (PR-2), peroxidase (PR-9), and total soluble protein were estimated at 7, 21, and 35 days after germination. These resistance-inducing chemicals work via systemic acquired resistance, an eco-safe technique providing long-term protection. Our results revealed that SA and BABA significantly increased PR-proteins' activities viz. chitinase,  $\beta$ -1,3-glucanase, peroxidase and total soluble protein content compared to the controls. Furthermore, treated plants showed a remarkable reduction in cotton leaf curl disease incidence and index compared to untreated plants. The higher activity of PR-proteins might be responsible for the decreased disease incidence and index in cotton cultivars. In addition,  $\beta$ -1,3-glucanase and chitinase negatively correlated with disease incidence and index, strongly indicating PR-proteins' role in the plant defense mechanism. A comparison between modes of elicitor treatment indicates seed priming to be the most effective as seed treatment given once sustained its effect up to 35 DAG whereas foliar spray in general gave better results. Although both elicitors seemed at par in their effectiveness, economy-wise SA will be the preferred elicitor. Therefore, SA treatment is suggested as an effective and eco-safe method to induce resistance against cotton leaf curl disease.

**Keywords:**  $\beta$ -aminobutyric acid, Cotton leaf curl disease, Salicylic acid, Systemic acquire resistance, Pathogen-related protein

Cotton is one of the most important cash crops and is also known as white gold. China, India, the United States, Pakistan, Türkiye, Brazil and Uzbekistan are major cotton-growing countries that contribute more than 84% of the total cotton production around the globe (Akin 2024). According to ICAC Journal 'Cotton This Month' in June 2023 the worldwide production of cotton was approximately 24.51 million tonnes (144.1 million bales) from 32.41 million hectares with productivity of 756 Kgs/hectare (Anonymous 2023). India holds 1<sup>st</sup> position in the world in cotton cultivation area of 12.7 million hectares with a production of 5.84 million tonnes and an average productivity of 447 Kgs/hectare (Anonymous 2023 and Keelery 2024). Cotton leaf curl disease (CLCuD), caused by a *Geminivirus* belonging to the family *Geminiviridae*, genus *Begomovirus*, is a severe and constant threat to cotton growers worldwide (Sattar et al., 2013). The DNA-1/DNA-A/DNA- $\beta$  complex of *Begomovirus* is responsible for CLCuD. The whitefly's spreading vector is difficult to control due to the prevalence of multiple virulent viral strains or related species (Akhtar et al., 2010, Ullah et al., 2014, Zubair et al., 2017).

Several short-term (mostly management practices) and

long-term approaches (developing resistant cotton varieties) were formulated to control this disease. The use of resistant varieties is the safest, most economical and most effective option to manage, but unfortunately, introgressed host plant resistance was rapidly overcome by the resistant breeding strain of CLCuBuV during 2005 and all the available cultivated genotypes from *G. hirsutum* were susceptible (Akhtar et al., 2010). This situation highlights the urgent need to explore new approaches to manage this devastating disease effectively.

Currently, several new strategies are being investigated to control various diseases for example use of various synthetic and biological compounds like 2,6-dichloroisonicotinic acid (INA), benzothiadiazole (BTH), salicylic acid (SA), jasmonic acid (JA) and  $\beta$ -aminobutyric acid (BABA) called resistance inducing chemicals (RICs). RICs can regulate numerous plant diseases by activating systemic acquired resistance (SAR) (Kumari et al., 2020 a,b). This mechanism involves activating the plant's resistance through chemical or biological agents, offering a non-conventional and eco-friendly approach to plant protection (Stockwell 2004, Raikhel and Pirrung 2005). SAR induces

various PR-proteins such as PR-1,  $\beta$ -1,3-glucanase (PR-2), acidic and basic class III chitinases, hevein-like protein (PR-4), thaumatin-like or osmotin-like proteins (PR-5), among others, which function as antiviral, antifungal, antibacterial, insecticidal, and nematocidal agents (Ward et al., 1991). For instance, in TMV-infected tobacco plants, SAR genes code for various pathogenesis-related proteins, with  $\beta$ -1,3-glucanase and chitinase hydrolyzing the microorganism's cell wall made of glucan and chitin (Gozzo 2003). Peroxidase activation is crucial for cell wall reinforcement and serves as a marker of the induced state (Kumari et al., 2022). Numerous studies have demonstrated the effective use of RICs to trigger SAR and control viral diseases in plants (War et al., 2012, Gordy et al., 2015, Thakur et al., 2016, Raj et al., 2016, Kumari et al., 2020b). PR-proteins like  $\beta$ -1,3-glucanase and chitinase reduce TMV infection in tobacco plants (Sindelarova and Sindelar 2005, Kumari et al., 2020a).

Thus, keeping cognizance of the above, the current study, was undertaken to investigate the effects of different SA and BABA for induction of pathogenesis-related proteins against cotton leaf curl disease

## MATERIAL AND METHODS

### Experimental Design

The experiment was conducted on three cotton cultivars viz. susceptible (F1378) and moderately resistant (LH2076) and resistant (FDK124). The cotton seeds were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Before sowing seeds were treated with 250 $\mu$ M concentration of the different resistance-inducing chemicals viz, SA and BABA for seed priming. Followed by foliar spray of both SA and BABA with the same concentration was sprayed on three different periods 5, 9 and 21 days after germination (DAG). For control, seeds were imbibed in distilled H<sub>2</sub>O for 6 hrs. Samples were collected at 7, 21 and 35 DAG for biochemical analysis. After 35 DAG plants were transferred to cages and viruliferous whiteflies were released on them @ six whiteflies/ plant and plants were disturbed two times a day for uniform and overall manifestation of CLCuD. The data on disease incidence and disease index was collected at 20, 30, and 40 days after infestation (DAI).

### Extraction and Assay of Chitinase and $\beta$ -1, 3-Glucanase

100mg leaf sample was crushed in a pre-chilled pestle and mortar using glass capillaries, in ice-cold 0.1 M sodium acetate buffer (pH 5.0 containing 2-mercaptoethanol). The sample extract was centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant obtained after centrifugation was used as an enzyme extract for the estimation of chitinase and  $\beta$ -1, 3-Glucanase activities. The standard procedure by

Boller and Mauch (1988) and Kauffmann et al., (1987) was followed for the assay of Chitinase and  $\beta$ -1, 3-Glucanase.

### Extraction and Assay of Peroxidase (POD)

Plant samples (100mg) were crushed in ice-cold potassium phosphate buffer (0.1 M, pH 7.5 containing 1 mM EDTA, 1% PVP and 10 mM of 2-mercaptoethanol) and centrifuged at 10,000 rpm for 30 min. The supernatant obtained was used for enzyme assay of peroxidase. All operations were done at 4 °C and assayed at 25 °C  $\pm$  1. Standard procedure of Claiborne and Fridovic (1979) was used for estimation of peroxidase.

### Extraction and Assay of total soluble protein content

Standard procedure of Lowry et al., (1951) is used for estimation of total soluble protein.

**Disease incidence (%):** The per cent disease incidence was worked out using the formula:

$$\text{Disease Incidence (\%)} = \frac{P_i}{P_t} \times 100$$

Where,  $P_i$  = Number of infected plants  $P_t$  = Number of total plants

### Disease index (%)

$$\text{Disease index (\%)} = \frac{N_1}{S_1} \times \frac{S_2}{N_2} \times 100$$

Where,

$N_1$  = Number of plants in control

$N_2$  = Number of plants in test entry

$S_1$  = Sum of all infection grades in control

$S_2$  = Sum of all infection grades in test entry

Rating Symptoms  
scale

0	Plants free from CLCuD
1	Thickening of small veins, only a few upper leaves affected
2	Thickening of veins, curling and cupping of leaves
3	Thickening of veins, curling and cupping of leaves, enation development on the underside of leaves
4	Thickening of veins, cupping, enations, stunting of plants and few bolls

The plants were graded according to the revised CLCuD scale described by AICCIP (Anonymous 2008) as given below.

### Statistical Analysis

All the experiments were conducted in triplicates and presented as mean $\pm$ SD. Means were compared by using Duncan's Multiple Range test at  $p \leq 0.05$ . All analysis was done by using two ways of analysis variance (ANOVA) by SPSS software version 23.

## RESULTS AND DISCUSSION

The plant's defense mechanism is typically not based on only single biochemical components but depends upon the

whole spectrum of biochemical components (Tripathi et al., 2019). Systemic acquired resistance (SAR) is a distinctive and vital mechanism because of its possible implication in terms of eco-friendly techniques providing long-duration protection to the plant (Shine et al., 2019). In the SAR mechanism plant's resistance mechanism is activated by prior treatment of chemical or biological agents known as resistance-inducing chemicals (RICs) (Akram and Anjum 2011, Gaikwad and Balgude 2016). Exogenous application of RICs for instance SA and BABA leads to induction of SAR against the cotton leaf curl disease. In the present investigation, treatment with BABA through seed priming resulted in a significant increase in PR-protein compared to control. The highest average increase of  $\beta$ -1, 3 glucanase activity through seed priming was observed in susceptible genotypes F1378 (3.0-fold) followed by resistant genotypes FDK 124 (1.7-fold) as compared with control plants. However, via foliar spray of BABA enzymes activity either increased or remained the same till 35 DAG. In contrast, maximum induction in  $\beta$ -1, 3 glucanase activity was recorded at 35 DAG in moderately resistant genotypes LH2076 (1.2-fold) than the control (Table 1). RICs such as SA, BABA, JA and other organic compounds were reported for induced resistance through the SAR mechanism when applied as seed priming, foliar spray and soil drench (Gordy et al., 2015, Thakur et al., 2016, Kumari et al., 2020b). BABA is also registered to persuade resistance against several environmental stresses and plant pathogens including viruses, bacteria and fungi when applied as seed imbibition and foliar spray (Jakab et al., 2001, Cohen 2010, Walters et al., 2013). The possible mechanism of BABA is explained by Wang et al. (2019) in grape berries against *Botrytis cinerea*, BABA caused early H<sub>2</sub>O<sub>2</sub> burst which leads to the activation of various PR-proteins genes and accumulation of phytoalexins.

Treatment with SA via both modes that is seed treatment and foliar treatment exhibited a significant increase of  $\beta$ -1, 3 glucanase activity. The highest increase was observed in F1378 and FDK124 (1.6-fold) via seed priming. LH2076 and FDK124 (1.6-fold) showed the highest increment in enzyme activity with foliar spray as compared to control at all stages of plant development (Table 1). A previous study reported that exogenous application of SA and BABA via seed imbibition increases  $\beta$ -1,3-glucanase and chitinase activity in tomato plants providing defense against *Alternaria* leaf blight (Raut and Borkar 2014).

In current study application of RICs (BABA and SA) significantly increased the activity peroxidase in treated cotton plant compared to the untreated plants with both mode of application (Table 3). Seed priming with BABA exhibited

highest significant average increase of peroxidase activity was in F1378 (1.6-fold) as compared to untreated plants. Similarly, a comparable result was recorded with foliar spray which exhibited maximum significant increase was recorded in F1378 (1.7-fold) whereas lowest increases were LH2076 (1.4-fold) with BABA treatment at all stage of stages. However, seed priming with SA showed a significant increase of peroxidase activity in FDK124 (2.0-fold) followed by F1378 (1.5-fold) via seed priming. However, FDK124 (1.5-fold) and LH2076 (1.4-fold) showed the highest average increase in POX activity via foliar spray compared to control plants at all stages of sampling (Table 3). Similar results were also obtained by Thakur et al., (2016), that is foliar application of SA resulted in induction of peroxidase, polyphenol oxidase, protease inhibitor and amylase inhibitor with JA and SA in brassica genotypes might be help in strengthening of plant cell wall. Which is further negatively correlated with peroxidase and polyphenol oxidase leads reduction in aphid population. Peroxidases are involved in varied range of physiological processes such as strengthening of host plant cell walls by oxidation of phenolic compounds, lignification, suberization, auxins metabolism, wound healing associated with H<sub>2</sub>O<sub>2</sub> decomposition against pathogens and insect pest (Pandey et al., 2017). Peroxidase is also registered to be a significant controller of various disease regulatory responses which are accompanying with SAR or HR by using RICs viz. INA, SA, and BTH through seed imbibition, foliar spray and soil drench (Bacelli and Mauch-Mani 2016, Kumari et al., 2020b).

Chitinase enzyme was induced either local or systemic after pathogen attack or with elicitors treatment which might be responsible for defence mechanism in plants against various pathogens. Chitinase activity was significantly upregulated with SA and BABA when seed priming and foliar spray were applied at different sampling stages (Table 2). Application of BABA through seed priming resulted in the highest average increase of chitinase activity in moderately resistant genotype LH2076 (2.9-fold) and resistant genotype FDK124 (1.8-fold) in comparison to untreated plants. Induction of these PR-protein by seed imbibition with SA and acetylsalicylic acid (ASA) was also observed in different studies (Abd-El-Kareem et al., 2004, El-Mougy 2004, Gaikwad and Balgude 2016, Kumari et al., 2020a). Likewise, SA and BABA also reported an increase in chitinase activity in brinjal and harvested peaches (Mahesh et al., 2017, Wang et al., 2018). In the case of foliar spray with BABA, the maximum average increase was in FDK124 (2.5-fold) and LH2076 (2.3-fold) as compared to untreated plants. While seed priming with SA, the highest increase was in LH2076 (3.4-fold) and FDK124 (2.3-fold) whereas foliar spray caused a maximum

**Table 1.** Effect of 250  $\mu$ M of BABA and SA on  $\beta$ -1, 3-glucanase activity (mg Glucose released /min/g FW)through foliar spray and seed priming in cotton genotypes

Genotypes	Treatments	$\beta$ -1,3 Glucanase (mg glucose released /min/g FW)					
		Foliar spray			Seed priming		
		7 DAG	21 DAG	35 DAG	7 DAG	21 DAG	35 DAG
F1378	Control	1.45 <sup>c</sup>	1.57 <sup>e</sup>	1.67 <sup>g</sup>	1.45 <sup>cd</sup>	1.57 <sup>defg</sup>	1.67 <sup>def</sup>
	BABA	1.36 <sup>b</sup>	1.49 <sup>cd</sup>	1.66 <sup>fg</sup>	4.38 <sup>n</sup>	4.85 <sup>o</sup>	4.81 <sup>o</sup>
	SA	1.26 <sup>a</sup>	1.90 <sup>i</sup>	2.02 <sup>j</sup>	2.28 <sup>l</sup>	2.42 <sup>k</sup>	2.57 <sup>k</sup>
LH2076	Control	1.35 <sup>b</sup>	1.51 <sup>d</sup>	1.64 <sup>fg</sup>	1.35 <sup>l</sup>	1.51 <sup>bc</sup>	1.64 <sup>def</sup>
	BABA	1.22 <sup>a</sup>	1.48 <sup>cd</sup>	1.92 <sup>m</sup>	1.51 <sup>fg</sup>	1.66 <sup>def</sup>	1.94 <sup>g</sup>
	SA	2.23 <sup>k</sup>	2.34 <sup>l</sup>	2.51 <sup>fg</sup>	1.01 <sup>hi</sup>	1.59 <sup>fg</sup>	1.84 <sup>h</sup>
FDK124	Control	1.32 <sup>b</sup>	1.46 <sup>bc</sup>	1.63 <sup>cd</sup>	1.32 <sup>b</sup>	1.46 <sup>cde</sup>	1.63 <sup>fg</sup>
	BABA	1.48 <sup>cd</sup>	1.62 <sup>f</sup>	1.83 <sup>h</sup>	2.01 <sup>i</sup>	2.44 <sup>k</sup>	2.85 <sup>m</sup>
	SA	2.24 <sup>k</sup>	2.36 <sup>l</sup>	2.49 <sup>m</sup>	2.32 <sup>jk</sup>	2.42 <sup>k</sup>	2.59 <sup>j</sup>

Significant difference in peroxidase activity is indicated by Star in comparison to their respective control  $P \leq 0.05$  analyzed by Tukey 's post hoc test

**Table 2.** Effect of 250  $\mu$ M of BABA and SA on chitinase activity ( $\mu$ M NAG released/ min/g FW)through foliar spray and seed priming in cotton genotypes

Genotypes	Treatments	Chitinase activity ( $\mu$ M NAG released/ min/g FW)					
		Foliar spray			Seed priming		
		7 DAG	21 DAG	35 DAG	7 DAG	21 DAG	35 DAG
F1378	Control	0.53 <sup>ab</sup>	0.73 <sup>cd</sup>	1.11 <sup>fg</sup>	0.53 <sup>ab</sup>	0.73 <sup>cd</sup>	1.11 <sup>h</sup>
	BABA	0.80 <sup>d</sup>	1.2 <sup>gh</sup>	1.83 <sup>kl</sup>	0.74 <sup>d</sup>	1.04 <sup>gh</sup>	1.49 <sup>ij</sup>
	SA	1.24 <sup>h</sup>	1.61 <sup>j</sup>	2.11 <sup>m</sup>	1.45 <sup>i</sup>	1.57 <sup>k</sup>	1.61 <sup>k</sup>
LH2076	Control	0.45 <sup>a</sup>	0.61 <sup>b</sup>	0.93 <sup>e</sup>	0.45 <sup>a</sup>	0.61 <sup>b</sup>	0.93 <sup>ef</sup>
	BABA	0.93 <sup>e</sup>	1.42 <sup>j</sup>	2.27 <sup>no</sup>	1.11 <sup>h</sup>	2.03 <sup>l</sup>	2.69 <sup>n</sup>
	SA	1.07 <sup>f</sup>	1.91 <sup>l</sup>	2.36 <sup>o</sup>	0.99 <sup>fg</sup>	2.59 <sup>n</sup>	3.27 <sup>o</sup>
FDK124	Control	0.49 <sup>a</sup>	0.63 <sup>bc</sup>	0.93 <sup>e</sup>	0.49 <sup>a</sup>	0.63 <sup>bc</sup>	0.93 <sup>ef</sup>
	BABA	1.15 <sup>gh</sup>	1.78 <sup>k</sup>	2.19 <sup>mn</sup>	0.62 <sup>b</sup>	1.04 <sup>gh</sup>	2.28 <sup>m</sup>
	SA	0.96 <sup>e</sup>	1.82 <sup>kl</sup>	2.7 <sup>p</sup>	0.87 <sup>e</sup>	1.64 <sup>k</sup>	2.38 <sup>m</sup>

Significant difference in peroxidase activity is indicated by Star in comparison to their respective control  $P \leq 0.05$  analyzed by Tukey 's post hoc test

**Table 3.** Effect of 250  $\mu$ M of BABA and SA on peroxidase activity ( $\Delta$ /min/g FW) through foliar spray and seed priming in three cotton genotypes

Genotypes	Treatments	Peroxidase activity ( $\Delta$ /min/g FW)					
		Foliar spray			Seed priming		
		7 DAG	21 DAG	35 DAG	7 DAG	21 DAG	35 DAG
F1378	Control	1.42 <sup>a</sup>	2.52 <sup>cd</sup>	3.23 <sup>f</sup>	1.42 <sup>a</sup>	2.52 <sup>bc</sup>	3.23 <sup>de</sup>
	BABA	2.53 <sup>cd</sup>	3.79 <sup>jk</sup>	6.04 <sup>n</sup>	4.14 <sup>i</sup>	3.64 <sup>hij</sup>	3.8 <sup>jk</sup>
	SA	1.72 <sup>b</sup>	2.79 <sup>e</sup>	4.18 <sup>l</sup>	3.33 <sup>defg</sup>	3.81 <sup>jk</sup>	4.10 <sup>l</sup>
LH2076	Control	2.75 <sup>de</sup>	3.28 <sup>fg</sup>	3.65 <sup>hij</sup>	2.75 <sup>c</sup>	3.28 <sup>def</sup>	3.65 <sup>hij</sup>
	BABA	3.49 <sup>gh</sup>	4.03 <sup>kl</sup>	6.26 <sup>n</sup>	3.23 <sup>de</sup>	3.62 <sup>ghij</sup>	4.02 <sup>kl</sup>
	SA	3.52 <sup>gh</sup>	4.13 <sup>kl</sup>	5.99 <sup>n</sup>	3.4 <sup>efgh</sup>	3.53 <sup>ghij</sup>	3.9 <sup>kl</sup>
FDK124	Control	2.31 <sup>bcd</sup>	3.05 <sup>efg</sup>	3.53 <sup>ghi</sup>	2.31 <sup>b</sup>	3.05 <sup>d</sup>	3.53 <sup>ghi</sup>
	BABA	3.68 <sup>hijk</sup>	3.85 <sup>kl</sup>	5.66 <sup>m</sup>	2.56 <sup>bcd</sup>	3.25 <sup>def</sup>	3.72 <sup>ij</sup>
	SA	3.22 <sup>f</sup>	4.05 <sup>kl</sup>	5.67 <sup>m</sup>	5.33 <sup>m</sup>	5.97 <sup>n</sup>	6.53 <sup>o</sup>

Significant difference in peroxidase activity is indicated by Star in comparison to their respective control  $P \leq 0.05$  analyzed by Tukey 's post hoc test

average increment in FDK124 (2.7fold) and LH2076 (2.6-fold) as compared to untreated plants. In addition, a significant and positive correlation was also observed between  $\beta$ -1, 3 glucanase and chitinase suggesting their defensive role in cotton against CLCuD (Table 6).

SA is a small phenolic molecule that induces SAR by activating different defence processes at basal and molecular levels and should be considered as a possible chemical control for the pest/pathogen (Lu et al., 2016) (Fig. 1). Tripathi et al. (2019) documented that phenyl ammonia lyase pathways mainly synthesize the SA which acts as a systemic signal in SAR and is the main reason for plant defence mechanisms under adverse conditions. Various plant protein serves as viral suppress and mediate several numbers of defence mechanisms. Total soluble protein was increased in all three cultivars after being treated with BABA and SA by seed priming and foliar spray (Table 4). The highest average protein content was in FDK124 (1.8-fold) with BABA when applied through seed priming. A similar trend was observed with SA which caused a 1.8-fold increase in total soluble protein content. In foliar spray, maximum increase was recorded with F1378 (1.7-fold) in comparison to

untreated plants. SA treatment resulted in a maximum average increase in total soluble protein in FDK124 (1.8-fold) via seed priming whereas LH2076 (1.6-fold) showed maximum increment via foliar spray compared to untreated plants at all three stages of sampling. Application of SA and BABA with seed priming and foliar spray increased total soluble protein at all development stages is might be due to the increased concentration of PR-protein in treated cotton plants. Proteins play a vital role in plant defense mechanisms in the form of many defense enzymes and other protein-based nonenzymatic compounds (War et al., 2011, Kumari et al., 2020a). Earlier studies reported upregulation of total soluble protein due to SA and JA might be due to an increase in PR-protein in brassica and cotton genotypes (Thakur et al., 2016, Raj et al., 2016). It is also documented that external application SA induced the total soluble proteins compared to untreated plants which provide resistance against tobacco necrosis virus (TNV) (Faheed and Mahmoud 2006). El-Khallal (2007) also recognized an increase in total soluble protein in tomato plants when treated with SA and jasmonic acid. When *B. napus* and *B. Juncea* were sprayed with SA and BTH increased total soluble protein. It was reported that

**Table 4.** Effect of 250  $\mu$ M of BABA and SA on chitinase activity ( $\mu$ M NAG released/ min/g FW) through foliar spray and seed priming in cotton genotypes

Genotypes	Treatments	Total soluble protein activity ( $\mu$ mol min <sup>-1</sup> mg <sup>-1</sup> FW)					
		Foliar spray			Seed priming		
		7 DAG	21 DAG	35 DAG	7 DAG	21 DAG	35 DAG
F1378	Control	7.48 <sup>c</sup>	8.80 <sup>e</sup>	10.28 <sup>h</sup>	7.48 <sup>b</sup>	8.80 <sup>c</sup>	10.28 <sup>e</sup>
	BABA	9.51 <sup>i</sup>	11.23 <sup>n</sup>	15.38 <sup>o</sup>	9.51 <sup>d</sup>	11.23 <sup>f</sup>	15.38 <sup>m</sup>
	SA	9.56 <sup>f</sup>	12.36 <sup>i</sup>	13.44 <sup>j</sup>	9.56 <sup>d</sup>	12.36 <sup>h</sup>	13.44 <sup>j</sup>
LH2076	Control	8.51 <sup>e</sup>	8.68 <sup>e</sup>	9.43 <sup>g</sup>	8.51 <sup>c</sup>	8.68 <sup>c</sup>	9.43 <sup>d</sup>
	BABA	10.11 <sup>h</sup>	11.93 <sup>j</sup>	14.35 <sup>l</sup>	10.11 <sup>e</sup>	11.93 <sup>g</sup>	14.35 <sup>i</sup>
	SA	11.78 <sup>k</sup>	12.85 <sup>l</sup>	16.21 <sup>n</sup>	11.78 <sup>g</sup>	12.8 <sup>i</sup>	16.21 <sup>n</sup>
FDK124	Control	6.42 <sup>b</sup>	7.53 <sup>c</sup>	9.61 <sup>g</sup>	6.42 <sup>a</sup>	7.53 <sup>b</sup>	9.61 <sup>d</sup>
	BABA	12.76 <sup>i</sup>	14.46 <sup>l</sup>	15.23 <sup>m</sup>	12.76 <sup>hi</sup>	14.46 <sup>l</sup>	15.23 <sup>m</sup>
	SA	12.46 <sup>a</sup>	13.88 <sup>b</sup>	15.13 <sup>d</sup>	12.46 <sup>hi</sup>	13.88 <sup>k</sup>	15.13 <sup>m</sup>

Significant difference in peroxidase activity is indicated by Star in comparison to their respective control  $P \leq 0.05$  analyzed by Tukey's post hoc test

**Table 5.** Effect of seed priming and foliar spray of SA and BABA@ 250  $\mu$ M on disease incidence and disease index

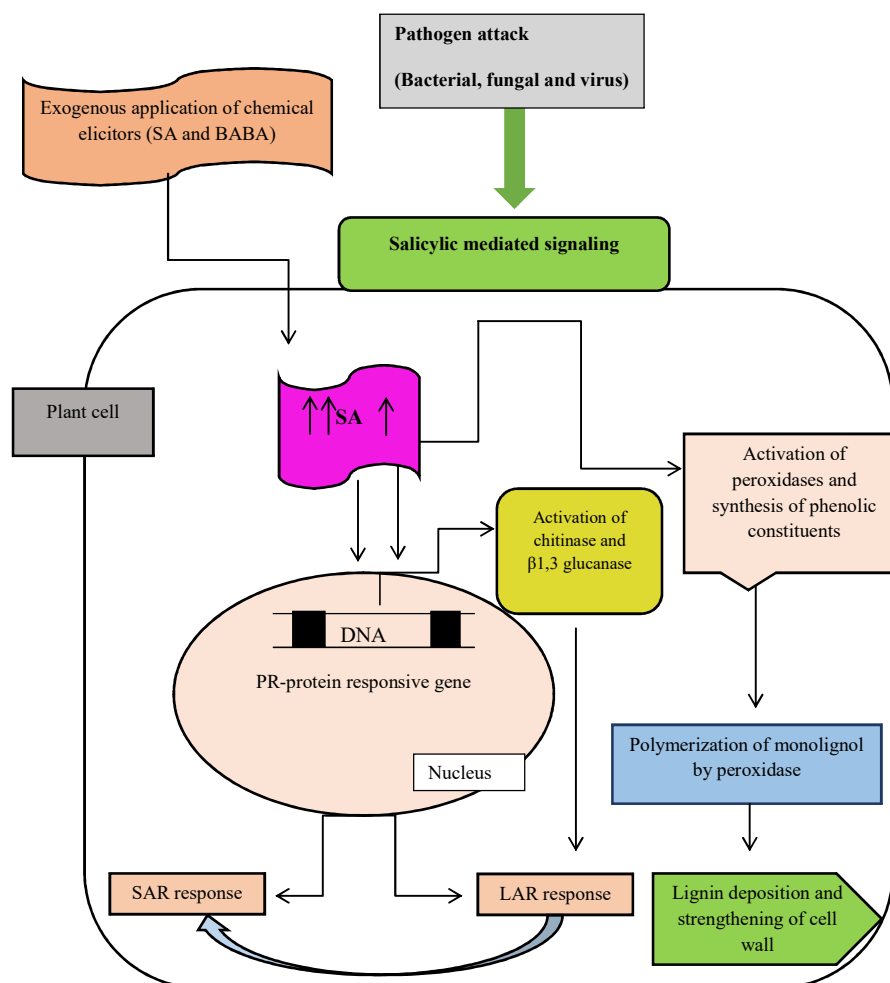
Cultivar	Disease Incidence (%)					Disease Incidence (%)					Disease Index (%)				
	30 DAI			30 DAI		40 DAI			40DAI		Seed priming			Foliar spray	
	Con	SA	BABA	SA	BABA	Con	SA	BABA	SA	BABA	Con	SA	BABA	SA	BABA
F1378	34	17	0	20	14	67	28	28	34	29	75	40	50	53	60
LH2076	17	0	0	10	17	34	17	20	14	25	62	45	44	52	57
FDK124	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Con= Control, SA=salicylic acid, BABA=  $\beta$ -aminobutyric acid, DAI= day after infestation

the combined effect of SA + BTH exhibited higher total soluble protein content in both genotypes after each spray (Thakur and Sohal 2014).

**Disease incidence and disease index:** Both SA and BABA showed decreases in disease incidence and index (Table 1). No disease symptoms were observed upto 20 DAI on the

cultivars. In cucumber seedlings, *Fusarium* blight disease was reduced by up to 66% when seeds were imbibed with SA (Wisniewska and Chelkowski 199). In cacao, SA was applied as seed imbibition, and foliar spray resulted in the reduction of *Phytophthora palmivora* infection. The resistant cultivar *i.e* FDK124 showed zero disease incidence and disease index.



**Fig. 1.** SAR by activating different defense process at basal and molecular level considered as a possible chemical control for the pest/pathogen

**Table 6.** Correlation between BABA treated biochemical parameters disease incidence and disease index

	β-1,3 glucanase	Chitinase	Peroxidase	Total soluble protein	Disease incidence	Disease Index
β-1,3 glucanase	1					
Chitinase	0.73792*	1				
Peroxidase	-0.24870	0.56281*	1			
Total soluble protein	-0.24156	0.43767	0.21614	1		
Disease incidence	-0.57225*	0.09012	-0.12024	-0.42414*	1	
Disease Index	-0.02049	-0.54673*	0.34113	0.58509	-0.61895*	1

\*Correlation is significant at the 0.05 level, \*\* Correlation is significant at the 0.01 level

Susceptible genotypes were recorded with more severe disease symptoms compared to moderately resistant cultivars. In treated plants, susceptible genotypes F1378 showed 28% increases in disease incidence with both modes of application. A moderately resistant genotype recorded with 20 and 25% disease incidence via seed priming and foliar spray respectively at 40 DAI. Disease index for susceptible cultivars was 40 - 53% whereas it was 50-60% for moderately resistant cultivars it ranges from 45-52 and 44-57% with SA and BABA via seed priming and foliar spray, at 30 DAI to 40 DAI respectively. Our results also recorded with lower disease incidence and index with 250µM concentration of both RICs via seed priming and foliar spray as compared to untreated plants (Table 5). This lower disease incidence and index could be explained based on the upregulation of PR-protein after RIC treatment (Raj et al., 2016). These scientists reported that implementation of MeJA and JA led to the upregulation of different PR-proteins which induced the resistance against *Monilinia fructicola* and CLCuD in peach and cotton genotypes. Similarly, Kumari et al. (2020a) also documented the induction of different PR-protein and reduction of CLCuD with INA via three modes of application that is seed priming, foliar spray, and soil drench.

### CONCLUSION

Seed priming and foliar spray of SA and BABA induced the activities of PR-proteins,  $\beta$ -1,3 glucanase, peroxidase, chitinase as well as total soluble protein content in all tested cotton cultivars during the different stages of sampling. Increase in PR-proteins activity caused induction of SAR which might be responsible for lower disease incidence and disease index in cotton. Among the two methods, seed priming was more effective in term of higher PR-protein activities and total protein as it was done once in the plant's life sustained its impact upto 35 DAG. However, among the resistance inducing chemical SA was more eco-safe elicitor can be tried as protective regime against CLCuD. This study has scope for future in term of authentication of the result and application on large scale in crop field.

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Received 14 September, 2024; Accepted 30 December, 2024





## ***In vitro* Evaluation of Different Nutritive Media for Mycelia Growth of *Pleurotus eryngii* (DC ex Fr.) Quel**

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**Abstract:** The study was conducted to evaluate the effect of different solid and liquid media for the mycelial growth, cultural characteristics and growth rate of *Pleurotus eryngii*. Five different solid media viz., Potato dextrose agar (PDA), Carrot extract agar (CEA), Malt extract agar (MEA), Asthana and Hawker's agar (A&HA) and Czapek Dox Agar (CDA) as well as their broths were evaluated *in vitro* to find out the best nutritive media for the optimum mycelial growth of *Pleurotus eryngii*. Maximum diametric growth (56.27 mm) was in Potato dextrose agar followed by Malt extract agar (48.58 mm) and Asthana and Hawker's agar (41.84 mm). In liquid media, highest biomass (467.50 mg) was in Potato dextrose broth followed by Carrot extract broth (228.33 mg) and Malt extract broth (186.67 mg). The mycelial growth in different media was white, cottony growth having concentric rings or ray like pattern whereas, in Asthana and Hawker's as well as Czapek Dox agar, the mycelial growth was transparent white.

**Keywords:** *Pleurotus eryngii*, Mycelial growth, Nutrient media, Mushrooms

Among all the species of mushroom grown in the world, oyster mushrooms represent the third largest group of edible mushrooms. *Pleurotus* species are considered as a good source of protein, fibre, carbohydrates, vitamins and minerals, and are low in calories, fats and sodium content (Cohen *et al.*, 2002). Among commercially cultivated species of oyster, King oyster mushroom (*Pleurotus eryngii*) belongs to phylum Basidiomycota, order Agaricales, family *Pleurotaceae*, genus *Pleurotus* and species *eryngii* (Kang 2004). Due to the excellent consistency of the cap and stem, cooking qualities and longest shelf life, this mushroom is regarded as one of the best among all the species of *Pleurotus*. The longer shelf life is due to the less content of water and firm flesh of the sporocarp (Moonmoon *et al.*, 2010; Yildiz *et al.*, 2002). It is commonly known as 'Afghani dhingri' or 'King trumpet mushroom' as it forms the largest fruiting bodies among the oyster species. Also, as its fruit bodies taste like almond and abalone; it is known as 'Almond abalone mushroom'. This species is characterized by its cream to ochraceous brown fruiting bodies with flabelliform to depressed pileus and eccentric to lateral stipe. Every organism needs nutrients as a source of energy and certain environmental conditions for its growth and development. For successful cultivation of mushrooms, growth medium plays a very vital role because it provides necessary nutrients for proper growth of mycelium. The first vital stage towards the success of spawn production is the maintenance and revival of pure culture mycelium with splendid quality (Kumar *et al.*, 2018). All the microorganisms require a set of

conditions under which they can flourish and sporulate best where culture medium is the major factor which influences the fungal cultivation (Dhingra and Sinclair 2014). In India, the cultivation of this mushroom is yet to be explored. Therefore, the present study was aimed out to determine the best nutritive media for *in vitro* cultivation of *Pleurotus eryngii* under subtropical zone of Himachal Pradesh.

### **MATERIAL AND METHODS**

The current study was carried out in the department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, India.

**Procurement, maintenance and preservation of the culture:** The pure culture of King oyster mushroom (*Pleurotus eryngii*) strain DMRP-135 was procured from Directorate of Mushroom Research, ICAR Complex, Chambhaghat, Solan. The culture thus obtained was maintained on potato dextrose agar (PDA) medium and sub-cultured periodically at an interval of 45 days. The full-grown culture was stored in the refrigerator at 2-4°C and was used for further studies.

**Sterilization of the glassware and culture media:** All media under study were sterilized in an autoclave at 121.5°C temperature and 15 psi pressure for 20 minutes. Glassware was sterilized in hot air oven at 180°C for 2 hours. The inoculating needle and cork borer were initially dipped in ethyl alcohol, flame sterilized and used after complete cooling.

**Cultural studies:** *In vitro* studies were carried out to

determine the best nutritive media for the growth of *Pleurotus eryngii* as per the standard method given by Lilly and Barnett (1951).

**Screening of solid and liquid media:** Five different solid media viz., potato dextrose agar (PDA), malt extract agar (MEA), carrot extract agar (CEA), Czapek's dox agar (CDA) and Asthana and Hawker's agar (A&HA) were evaluated for mycelial growth of *P. eryngii*. With the help of a sterilized cork borer, mycelial bits of 5.0 mm diameter were cut from the actively growing areas of pure culture plate and inoculated in the centre of Petri plate of the respective media and then incubated in a BOD incubator at 25±1°C. Data were recorded in terms of average diametric growth (mm), cultural characteristics (type of growth and colour of mycelium) and growth rate (mm/h) up to 240 h of incubation. Growth rate (mm/h) was calculated with the help of following formula:

$$r_g = \frac{dgt_2 - dgt_1}{t_2 - t_1}$$

where,  $r_g$  = growth rate (mm/h),  $dgt_2$  = Diametric growth (mm) at time  $t_2$ ,  $dgt_1$  = Diametric growth (mm) at time  $t_1$

Five different liquid media potato dextrose broth (PDB), malt extract broth (MEB), carrot extract broth (CEB), Czapek's dox broth (CDB) and Asthana and Hawker's broth (A&HB) were evaluated for mycelial growth of *P. eryngii*. A 5.0 mm diameter bit of test fungus was taken with the help of sterilized cork borer from the pure culture plate and inoculated in respective broth of 75 ml (150 ml capacity Erlenmeyer's flasks). After inoculation, the flasks were incubated in a BOD incubator at 25±1°C. Data were recorded in terms of dry mycelial weight (mg) after 7, 14 and 21 days of inoculation. For determination of fungal biomass in liquid media, mycelial mat of the test fungus was filtered through Whatman's No. 1 filter paper disc and dried at 50°C overnight. The dry weight of the fungus was calculated by using the following formula:

Dry weight of the fungus = (weight of filter paper + mycelium) – (weight of filter paper)

**Data analysis:** The experiments were conducted in completely randomized design with four replications in each treatment and statistically analysed by using statistics package program OPSTAT (Sheoran, 2006).

## RESULTS AND DISCUSSIONS

**Effect of different solid media on the growth of *Pleurotus eryngii*:** Significantly mean maximum (56.27 mm) diametric growth was in PDA followed by MEA (48.58 mm) while, mean minimum (32.36 mm) was in CEA (Table 1). Irrespective of different media under study, significantly mean maximum (70.85 mm) diametric growth was after 240 h of incubation followed by 192 h (61.30 mm) while, mean minimum (12.03 mm) diametric growth was recorded after 48 h of incubation (Plate 1). Significantly maximum (89.64 mm) diametric growth was in PDA after 240 h of incubation followed by 192 h (80.65 mm) on same medium while, minimum (10.13 mm) diametric growth was in CEA after 48 h of incubation which was statistically at par with CDA (10.19 mm) after same duration of incubation. Rest of the treatments exhibited intermediate level of diametric growth.

Colour of mycelium was white in PDA, MEA and CEA while transparent white in A&HA and CDA (Table 1). The thick cottony growth was in PDA while dense compact growth with ray like pattern in MEA. In CEA, fluffy growth having concentric rings was recorded with progression of time while, thin and transparent growth was recorded in A&HA and CDA. Significantly mean maximum growth rate (0.35 mm/h) was in PDA followed by MEA (0.30 mm/h) and A&HA (0.26 mm/h) (Table 2). However, significantly mean minimum growth rate (0.21 mm/h) was in CEA. Irrespective of different nutrient media under investigation, significantly mean maximum growth rate (0.40 mm/h) was between 96-144 h of incubation

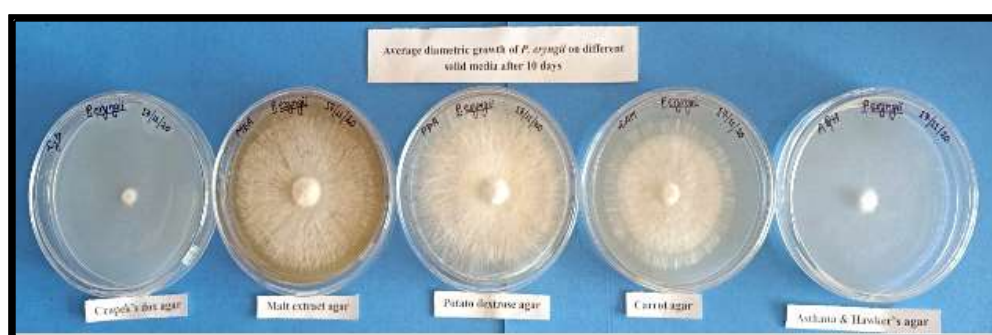
**Table 1.** Effect of different solid media on mycelial growth and mycelial characteristics of *Pleurotus eryngii*

Nutrient medium	Average diametric growth (mm) after different duration of incubation (h)					Overall mean	Colour of mycelium	Type of growth
	48	96	144	192	240			
Potato dextrose agar	13.38	35.32	62.38	80.65	89.64	56.27	White	Thick cottony
Malt extract agar	15.31	33.38	51.64	66.19	76.38	48.58	White	Dense compact growth having ray-pattern
Carrot extract agar	10.13	19.57	31.90	45.06	55.13	32.36	White	Fluffy growth having concentric rings
Asthana and Hawker's agar	11.13	26.22	45.75	58.54	67.56	41.84	White	Thin, transparent growth
Czapek's dox agar	10.19	23.82	41.14	56.06	65.57	39.35	White	Thin, transparent growth
Overall mean	12.03	27.66	46.56	61.30	70.85			
CD <sub>p=0.05</sub>	Medium 0.08	Duration 0.08	Interaction 0.19					

followed by 48-96 h (0.33 mm/h) while, significantly mean minimum growth rate (0.15 mm/h) was between 0-48 h of incubation. Interaction between nutrient media and time of incubation reveals that maximum average growth rate (0.56 mm/h) of the test fungus was in PDA between 96-144 h of incubation followed by 48-96 h (0.46 mm/h) on same medium. However, minimum average growth rate (0.11 mm/h) was in CEA and CDA between 0-48 h of incubation which was statistically at par with A&HA(0.13 mm/h) between same duration of incubation. Rest of the treatments exhibited intermediate levels of growth rate.

**Effect of different liquid media on mycelial growth of**

***Pleurotus eryngii***: irrespective of different durations of incubation, significantly maximum (467.50 mg) biomass was in PDB followed by CEB (228.33 mg) and MEB (186.67 mg) (Table 3). However, significantly mean minimum (87.50 mg) biomass of the test fungus was in A&HB. Irrespective of different liquid media under study, significantly mean maximum (295.00 mg) biomass was recorded after 21 days of incubation followed by 14 days (237.00 mg) while, mean minimum (147.50 mg) biomass was recorded after 7 days of incubation (Plate 2). Interaction between nutrient broth and time interval reveals that significantly maximum average biomass (577.50 mg) was recorded in PDA after 21 days of



**Plate 1.** Mycelial growth of *Pleurotus eryngii* on different solid media

**Table 2.** Effect of different solid media on growth rate of *Pleurotus eryngii*

Nutrient medium	Growth rate (mm/h) between different duration of incubation (h)					Overall mean
	0-48	48-96	96-144	144-192	192-240	
Potato dextrose agar	0.18	0.46	0.56	0.38	0.19	0.35
Malt extract agar	0.21	0.38	0.39	0.31	0.21	0.30
Carrot extract agar	0.11	0.20	0.26	0.27	0.21	0.21
Asthana and Hawker's agar	0.13	0.32	0.41	0.27	0.19	0.26
Czapek's dox agar	0.11	0.29	0.36	0.31	0.20	0.25
Overall mean	0.15	0.33	0.40	0.31	0.20	
CD (p=0.05)	Nutrient medium 0.01		Duration 0.01		Interaction 0.03	

**Table 3.** Effect of different liquid media on biomass production of *Pleurotus eryngii*

Nutrient medium	Average dry weight (mg) after different duration of incubation (Days)			Overall mean
	7	14	21	
Potato dextrose broth	360.00	465.00	577.50	467.50
Malt extract broth	132.50	180.00	247.50	186.67
Carrot extract broth	135.00	242.50	307.50	228.33
Asthana and Hawker's broth	60.00	80.00	122.50	87.50
Czapek's dox broth	50.00	217.50	220.00	162.50
Overall Mean	147.50	237.00	295.00	
CD (p=0.05)	Nutrient broth 17.53	Duration 13.58	Interaction 30.37	



**Plate 2.** Effect of different liquid media on biomass production of *Pleurotus eryngii*

inoculation followed by 465.00 mg after 14 days and 360.00 mg after 7 days of inoculation in the same medium. However, significantly minimum average biomass (50.00 mg) of the test fungus was recorded in CDB after 7 days of inoculation which was statistically at par with A&HB (60.00 mg) after 7 days of inoculation and 14 days of incubation (80.00 mg). Rest of the treatments exhibited intermediate levels of biomass production.

Shrestha et al. (2006) observed that media which is rich in nutrients produced mycelium in abundance. This may be due to the sufficiency of all the nutritional requirements and optimal physical conditions for the vegetative growth of mycelium. Sardar et al. (2015) also reported maximum growth rate (0.52 cm/day) of *Pleurotus* spp. including *P. eryngii* in PDA medium. Nguyen and Ranamukhaarachchi (2020) observed maximum mycelial growth and growth rate (5.89 cm and 0.84 cm/day) of *P. eryngii* in potato dextrose agar medium. Different workers also reported potato glucose liquid medium and potato dextrose broth as the best medium for maximum biomass production of different species of *Pleurotus* (Diwan and Rawte 2011, Abd El Zaher et al., 2020).

### CONCLUSION

Potato dextrose agar medium exhibited maximum mycelial growth followed by malt extract agar medium. Mean minimum mycelial growth was observed in carrot extract agar medium. Growth rate was maximum in potato dextrose medium followed by malt extract agar while was least in carrot extract agar medium. Mean maximum biomass was observed in potato dextrose broth followed by carrot extract broth and malt extract broth. However, mean minimum biomass was recorded in Asthana and Hawker's broth. In all the media under study, the colour of the mycelium varied from white to transparent white. The type of growth was observed

as cottony, fluffy, thin and transparent having ray pattern and concentric rings.

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# Introgression of Banded Leaf and Sheath Blight (BLSB) Resistance from Teosinte to Maize Cultivar

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**Abstract:** Maize (*Zea mays* L.) is a member of the *Poaceae* family and is targeted as the world's most important strategic cereal food crop. This study was planned to screen the maize-Teosinte RILs population for banded leaf and sheath blight and agronomic traits. A total of 338 maize-Teosinte recombinant inbred lines (RILs), derived from cross between the high popping volume (HPV) Canadian popcorn inbred line (susceptible) as the female parent and Teosinte (wild relative) as the male parent (resistant). The data were recorded for agronomic traits and disease score for banded leaf and sheath blight. The mean sum of squares due to genotypes were observed highly significant ( $p < 0.01$ ) for all the trait studied. The 100 seed weight, an important trait showed highly significant positive correlation with number of seed rows per cob and cobs length. Path coefficient analysis revealed direct positive effect of cob length, number of seed rows per cob and plant height on grain yield. The first two Principal component values used to construct biplot graphs explained 39.70% of the total variation. The four maize-Teosinte RIL lines viz.; RIL-210, RIL-272, RIL-314 and RIL473 were screened resistant to banded leaf and sheath blight, which is a good source of BLSB resistance and would be utilized in breeding for maize improvement program. The recombinant inbred lines viz., RIL-6, RIL-26 and RIL-419 were identified as a better-performing line for multiple agronomic traits which, would also utilized to improve agronomic performance of maize.

**Keywords:** Recombinant inbred lines, Banded Leaf and sheath blight, *Rhizoctonia solani*, Popcorn, *Zea mays* L.

Maize (*Zea mays* L.) is a member of the *Poaceae* family and is the third most important cereal grain worldwide, after wheat and rice (Farrell and Hodges 2004) and targeted as the world's most significant strategic cereal food crop (Tefera 2020). It is a staple food crop for many countries of the world, including Latin America, Asia and Africa. Maize is grown on 197 million (M) hectares, with 32% grown in lower capacity income countries (Erenstein et al., 2021). Banded leaf and sheath blight (BLSB) caused by *Rhizoctonia solani* f. sp. *Sasakii* (Khunn) is a soil-borne pathogen having wide-ranging outbreaks in several portions of India (Singh et al., 2014). The banded leaf and sheath blight (BLSB) pathogen was first reported by Bertus in Sri Lanka in 1927 and is name sclerotial disease (Singh and Shahi 2012). Disease losses are especially severe when the relative humidity is high and the plant population density is high. This disease primarily affects the first and second leaf sheaths above ground and then progresses upward to the ear leading to serious crop losses and is spreading to new areas due to intensive farming techniques. It has been gaining epiphytotic proportions in the previous decades due to its rising severity. In currently produced cultivars, BLSB has the capacity to yield losses of up to 40–70% (Singh et al. 2018). The pathogen can cause 7.6 to 64.8% grain yield loss, while in the ear rot stage of BLSB, grain loss might reach 100% in continuous rain condition (Kumar et al., 2021).

Maize originated from wild grass (*Zea mays* ssp. *parviglumis*) around 9000 years back in the Balsas region of southwest Mexico. Teosinte is very closely related to maize and are classified as *Z. mays* ssp. *Mexicana* (Schradler) Iltis, *Z. mays* ssp. *huehuetenangensis*, which cultivate in northern and central Mexico. Teosinte has certain superiority as insect resistance, disease resistance, saline-alkaline resistance, drought resistance, reproductive ability and multiple tillers and has been successfully used in maize breeding as an important source of favorable alleles (Wang et al., 2020). The abiotic stress tolerance, pest and disease resistance, quality, yield and fertility restoration and male sterility is an important for all crops and have been improved with crop wild relatives (Hajjar and Hodgkin 2007). The introgression lines can be used to incorporate the BLSB resistance to maize cultivars while also improving their morphological and yield performance of maize. Additional breeding initiatives can make advantage of these introgression lines in further breeding programmes.

## MATERIAL AND METHODS

The experimental material includes the total of 338 maize-Teosinte recombinant inbred lines (RILs)  $F_4$ , derived from cross between the high popping volume (HPV) Canadian popcorn inbred line (susceptible) as the female

parent and Teosinte (wild relative) as the male parent (resistant) were sown during *kharif* season, 2021 along with parents in the last week of May at the Eternal University experimental field, Machher, Baru Sahib, Himachal Pradesh, India. Each genotype was represented by a plot of two rows of 1m and row to row spacing was maintained at 40 cm. The observations were recorded for agro-morphological traits and disease reaction. The recorded data were statistically analyzed to performed following standard procedures for the estimation of components of genetic variation, with the help of the SAS software, version 9.1. SAS Institute, Inc. SAS user's guide (Clark and Kempthorne 1958).

**Banded leaf sheath blight inoculum:** The pathogen of banded leaf sheath blight (BLSB) was isolated from sclerotia collected from infected plants in previous year's research field. These sclerotia were cultured on potato dextrose agar (PDA) medium, purified and stored in laboratory for subsequent use in preparing artificial disease inoculation for field experiments. Unhulled sorghum grains were soaked in potable water for 24 hours, dispense 50g in 250ml Erlenmeyer flask after drained of excess water, and autoclaved at a 121°C (15 psi pressure) for 30 minutes. Fresh culture was prepared in petri dish using the stored BLSB culture and pathogen started growing on culture plate. The sterilized cork borer was used to aseptically homogenize a 5-6 days old pathogen growth collected from potato dextrose agar in 10 ml sterile water, and 5 ml of homogenized culture was utilized to seed each flask and incubated at 27 °C for 10-14 days. Grains were shaken at 3-4 days interval for identical growth of fungal on sorghum grains. After approximately two weeks of incubation, the inoculum was ready for use and stored, dried, at 15°C for future applications.

**Artificial inoculation for banded leaf sheath blight:** In addition to various method of artificial disease inoculation, maize plants were subjected to BLSB pathogen under field conditions following the standard procedure outlined by Yadav et al. (2023). Inoculation was done on 30-45 days old plants after sowing of maize. Inserting four to five grains coated with fungal growth and sclerotia bodies gently between the stalk's rind and inoculating leaf sheath of three leaves in each plant at the second, third, and fifth internode from the base of the plant stem. Regular irrigation was applied to sustain high humidity levels (>90%) during the disease's progression. Inoculation was replicated again after 10 days to avoid chances of failure of disease growth. After 35 days of inoculation, each maize line was scored for disease and classified into groups depending on their reactivity.

**Disease score:** A disease rating scale of 0 to 5 was used to assess symptoms such as sheath damage, early plant death,

stem breakage, rotten ears and stalk lesions (Jiang et al., 2020).

**Morphological observations:** During the *kharif* season 2021, observations were recorded on morphological traits viz; emergence to flowering (days), days to maturity (days), plant height (m), number of cobs per plant (numbers), number of tillers per plant (numbers), number of branches per plant, number of seed rows per cob, cob length (cm) and hundred-grain seed weight (g).

**Statistical analysis:** The recorded data was subjected to statistically analyzed, following standard procedures for the estimation of components of genetic variation, correlations among yield-related traits and principal component analysis through SAS software, version 9.1. SAS Institute, Inc. SAS.

## RESULTS AND DISCUSSION

The analysis of variance revealed significant differences among these RILs for all the traits indicates that the genotypes employed in this study differ with regard to the characters that opened a way to proceed for further improvement through simple selection and these genotypes can used for selection in maize improvement program (Table 1). The results were also supported by Singh et al. (2017), Adhikari et al. (2019) and Sahoo et al. (2021).

**Performance of RILs for agronomic traits:** Days to flowering emergence ranged from 54 to 101 days. The early flowering was recorded for the five lines viz. RIL-6, RIL-27, RIL-104, RIL-323, and RIL-378 with their parent Canadian popcorn and Teosinte. Days to maturity ranged from 99 to 150 days. The early maturity was observed in five lines viz.; RIL-23, RIL-28, RIL-46, RIL-47 and RIL-143 as compared to the parents. The plant height ranged from 1.20 to 3.63 (m). The short height was recorded for the five lines viz.; RIL-51, RIL-125, RIL-304, RIL-392 and RIL-443 with respect to parents. The numbers of tillers per plant ranged from 1 to 4 tillers. The high numbers of tillers per plant were recorded for RIL-68, RIL-228, RIL-308, RIL397 and RIL-479 with the parents. The number of branches per plant ranged from 0 to 5. The high number of branches per plant were observed in RILs viz.; RIL-272, RIL-428, RIL-429, RIL-272 and RIL-429 with respect to parents. Numbers of cobs per plant ranged from 1 to 22. The lines were having high number of cobs per plant were RIL-46, RIL-272, RIL-428, RIL-429 and RIL-482 with the parents. The number of seed rows per cob ranged from 2 to 8. The lines with more seed rows per cob were observed for RIL302, RIL-358, RIL-403, RIL-419 and RIL-441 as compared to parents. Cob length ranged from 4. to 23.67cm. The RILs were observed with high cob length was RIL-9, RIL-26, RIL-109, RIL-358, RIL-382 as compared to parents. The 100 seed weight was ranged from 4.5 to 21.37

g. The RILs were observed the high seed weight viz; RIL-6, RIL-37, RIL-188, RIL-231 and RIL-358 (Table 1). The high variance values indicate that the genes played a significant role in this variance, and selection can be very effective. The observed ranges of different morphological and yield traits in study are in general accordance with similar studies in maize (Chaurasia et al., 2020, Kumawat et al., 2020, Pranay et al., 2022, Reddy et al., 2022, Al-Rawi et al., 2024).

#### Correlations among agronomic traits in maize-Teosinte

**RIL population:** Phenotypic correlations are observed that for the maize RIL population, a total of 17 correlations were found significant, 14 correlation coefficients were to be highly significant and 3 to be significant (Table 2). The emergence to flowering days had a highly positively significant correlation with days to maturity, number of tillers per plant and highly negative significant correlation with number of rows per cob, 100 seed weight, while, negative significant correlation with cob length. Plant height was a highly positively significant correlation with number of branches per plant and number of cobs per plant. Similar findings were also observed earlier workers (Malik et al., 2005, Rafiq et al., 2010, Bekele et al., 2014). Number of tillers per plant had a highly positive

significant correlation with number of branches per plant and the number of cobs per plant whereas, number of tillers per plant had a highly significant negative correlation with the number of seed rows per cob. Number of branches per plant was highly positively associated with number of cobs per plant, while, a highly negative correlation with number of seed rows per cob and a significant negative correlation with cob length. Number of cobs per plant also have a significant negative correlation with number of seed rows per cob. The number of seed rows per cob recorded a highly significant positive correlation with cob length, and 100seed weight. Cob length was also showed that a highly significant correlation with 100 seed weight (Table 2). Some researchers reported similar observations (Hengu et al., 2017, Izzam et al., 2017, Chaurasia et al., 2020, Pranay et al., 2022), The researchers observed that plant height, ear height, ear length, ear diameter, grains per row, 100-seed weight, cob yield per plant and seed vigour index were significant and positively significantly inter-correlated with the grain yield per plant and these traits possess high yield potential in maize crop.

#### Path coefficient analysis for maize-Teosinte RILs for

**Table 1.** Analysis of variance and morphological parameters for various agronomic traits of maize-Teosinte RIL population

Source of Variation	DF	Days to Tassel initiation (Days)	Days to maturity (Days)	Plant height (m)	No. of tillers per plant (Number)	No. of branches per plant (Number)	No. of cobs per plant (Number)	No. of Seed rows per cob (Number)	Cob length (cm)	100 seed weight (g)
Replication	2	83.962	96.862	0.2535	0.98	2.344	27.5335	2.2515	4.3985	10.8625
Treatment	339	167.924**	193.724**	0.507**	1.96**	4.688**	55.067**	4.503**	8.797**	21.725**
Error	678	6.544	6.301	0.009	0.075	0.105	0.563	0.751	3.813	0.992
Mean		78.00	125.00	2.37	1.54	1.00	8.14	4.20	7.31	10.09
Minimum		54.00	99.00	1.20	1.00	0.00	1.00	2.00	4.33	4.50
Maximum		101.00	150.00	3.63	4.00	5.00	22.00	8.00	23.67	21.37

\*, \*\* - significant at 5 % and 1 % level respectively

**Table 2.** Correlation among the different agronomic traits in maize-Teosinte RIL population

Parameter	Days to maturity (Days)	Plant height (m)	No. of tillers per plant (Number)	No. of branch per plant (Number)	No. of cobs per plant (Number)	No. of seed rows per cob (Number)	Cob length (cm)	100 seed weight (g)
Emergence to flowering (days)	0.242**	0.054	0.145**	0.009	-0.009	-0.220**	-0.120*	-0.186**
Days to maturity		-0.044	0.050	-0.038	-0.031	-0.069	-0.020	-0.032
Plant height (m)			0.010	0.171**	0.147**	-0.064	0.004	0.019
Number of tillers per plant				0.157**	0.382**	-0.160**	-0.059	-0.030
Number. of branch per plant					0.580**	-0.163**	-0.125*	-0.004
Numberof cobs per plant						-0.119*	-0.089	0.011
Numberof seed rows per cob							0.324**	0.196**
Cobs length (cm)								0.217**

\*, \*\* - significant at 5 % and 1 % level respectively

**agro morphological traits:** The maximum positive direct effects of cob length, number of seed rows per cob and plant height were observed on 100 seed weight (Table 3). The negative direct effect of emergence to flowering was observed on 100 seed weight. The number of seed rows per cob and cob length had indirect effect on 100 seed weight. Jakhar et al. (2017), Chaurasia et al. (2020) and Pranay et al. (2022) had also observed same results working on maize. Aman et al. (2020) reported significant positive direct effects of ear height on yield per hectare in maize.

**Principal Component Analysis (PCA) for agronomic traits:** The PCA was performed for 9 agronomic traits of the maize-Teosinte RIL population. The correlation matrix reported 9 major components, which correlate to the number of traits. The variability of PCA concentrate is explained by the first five principal components. The first four PCs explained 63.3 % of the total variation, in which the contribution of PC1 was 22.50%, PC2 17.20, PC3, 12.50 and PC4 11.20 in the total divergence of the studied population. Significant PCs had Eigen values ranging from 2.027 (PC1) to 1.004 (PC4) (Table 4). PCAs displayed that the total genetic diversity was clearly distributed throughout the agromorphological traits. Murtadha et al. (2018), observed the genetic variation of maize genotypes was significantly influenced by variables such as days to maturity, ear height, ear diameter, ear length, grain rows per ear, grains per row, and yield per plant. The utility of PCA in selecting maize genotypes through the selection of features that significantly contribute to the genetic variation of maize germplasms was also highlighted by Mustafa et al. (2015). Based on the factor loading graph (Fig. 1), 100 seed weight is strongly correlated with cob length and number of rows per cob. The 100 seed weight is negatively correlated with the emergence of flowering, and days to maturity (Fig. 1). The plant height is also strongly correlated with number of cobs

per plant, number of branches per plant, number of tiller per plant and negatively correlated with days to flowering initiation or days to maturity. Similar findings have been also observed by other reports Malik et al. (2005), Rafiq et al. (2010), Bekele et al. (2014). Aman et al. (2020) reported that PCA had a significant and positive correlation with days to 50% tasselling, plant height, ear height, and 100-grain weight in maize.

**Screening of maize-Teosinte RIL population for banded leaf and sheath blight:** After 45 days of inoculation, data pertaining to BLSB adopting a disease scale of 0-5 scale. There are three types of ear rot, (i) infection before ear initiation, the ear cannot be developed and it is rudimentary, (ii)infection after ear initiation; the stalk fiber at the tip darkens, becomes caked-up, and hardens, resulting in poor grain filling, (iii) infection after grain filling; the kernels become light in weight, chaffy, and lusterless. The presence of light brown cottony mycelium on the plant ear, the presence of tiny and round black sclerotial, premature drying of ears, and caking of the ear sheath are characterized signs

**Table 4.** Eigen values (latent roots) and rotated component loadings (principal component traits of maize-Teosinte RILs)

Component	Eigen value	Proportion (%)	Cumulative (%)
PC1	2.03	22.50	22.50
PC2	1.54	17.20	39.70
PC3	1.12	12.50	52.10
PC4	1.00	11.20	63.30
PC5	0.84	9.30	72.60
PC6	0.79	8.80	81.40
PC7	0.67	7.40	88.80
PC8	0.64	7.10	96.00
PC9	0.36	4.00	100.00

**Table 3.** Path coefficient analysis for various agronomic traits in maize-Teosinte RIL population

Parameter	Emergence to flowering (Days)	Days to maturity (Days)	Plant height (m)	No. of tillers per plant (Number)	No. of branch per plant (Number)	No. of cobs per plant (Number)	No. of seed rows per cob (Number)	Cobs length (cm)	r value with 100 seed weight
Emergence to flowering (days)	-0.146	0.004	0.002	0.001	0.000	0.000	-0.027	-0.020	-0.186**
Days to maturity	-0.035	0.017	-0.001	0.000	-0.001	-0.001	-0.008	-0.003	-0.032
Plant height (m)	-0.008	-0.001	0.028	0.000	0.003	0.003	-0.008	0.001	0.019
Number of tillers per plant	-0.021	0.001	0.000	0.008	0.003	0.008	-0.019	-0.010	-0.030
Number. of branch per plant	-0.001	-0.001	0.005	0.001	0.020	0.012	-0.020	-0.021	-0.004
Number of cobs per plant	0.001	-0.001	0.004	0.003	0.012	0.020	-0.014	-0.015	0.011
Number of seed rows per cob	0.032	-0.001	-0.002	-0.001	-0.003	-0.002	0.120	0.053	0.196**
Cobs length (cm)	0.018	0.000	0.000	-0.001	-0.002	-0.002	0.039	0.165	0.217**

\*Significant at 5% level; \*\*Significant at 1% level; Bold figure indicates direct effect; Residual effect=0.91360



of ear rot that are also observed in the maize-Teosinte RIL population. Later on the infection was visible on the lower sheath that manifested spreads to the upper leaf sheaths, resulting in leaf sheath rot and whole leaf drying in the maize-Teosinte RILs population. The disease occurs on 40-45 days old plants during the pre-flowering stage of the plant, resulting in blighting and the death of the apical region of growing plant in the maize-Teosinte RIL population. Lesions and blotches cover the majority of the leaves, making alternating narrow purple or brown zones more visible has been recorded in the maize-Teosinte RILs population, which is a characteristic symptom of BLSB.

**Disease reactions in maize-Teosinte recombinant inbred lines:** The parent line Canadian popcorn (HPV) was highly susceptible having the disease score 5 while the Teosinte was immune with 0 disease score (Table 5). The disease reaction of the 338 recombinant inbred lines is presented in Table 5. Banded leaf sheath blight disease scores ranged

from 0.0 to 5.0. There was no immune line observed while, four RILs viz.; RIL-210, RIL-272, RIL-314 and RIL-473 were observed resistant with a disease score 1. Forty-seven RILs were recorded as a moderately resistant to BLSB with a disease score of 2. Sixty-three RILs were observed with moderate susceptible reaction to this disease having the disease score 3, 166 lines recorded susceptible as having disease score 4 and 58 lines showed high susceptibility for BLSB with the highest disease score 5 (Table 5). The BLSB was observed in Indian farming systems, affecting grain yield economic losses by 40-100% (Malik et al. 2018). The symptoms of maize leaf and sheath blight are very similar to those described by Rijal et al. (2007) and (Sagar and Bhusal 2019). Chen et al. (2013) observed the same results in the 282 maize inbred lines of disease resistance to BLSB, four moderately resistant inbred lines were found and there was no immune line was recorded. Another study involved twenty-eight maize genotypes were tested for the BLSB

**Table 5.** BLSB reaction in maize-Teosinte RIL population

Disease scoring	Disease rating scale	Reaction	No. of lines	RILs ID
0	0	I	-	-
1-10%	1.0	R	4	RIL-210, RIL-272, RIL-314, RIL-473
11-25%	2.0	MR	47	RIL-1, RIL-3, RIL-9, RIL-11, RIL-14, RIL-20, RIL-23, RIL-24, RIL-39, RIL-48, RIL-55, RIL-59, RIL-62, RIL-72, RIL-83, RIL-108, RIL-109, RIL-126, RIL-142, RIL-185, RIL-192, RIL-203, RIL-205, RIL-207, RIL-220, RIL-223, RIL-230, RIL-257, RIL-283, RIL-290, RIL-300, RIL-302, RIL-312, RIL-322, RIL-336, RIL-398, RIL-418, RIL-428, RIL-429, RIL-436, RIL-439, RIL-442, RIL-453, RIL-478, RIL-479, RIL-480, RIL-486
26-50%	3.0	MS	63	RIL-5, RIL-8, RIL-12, RIL-15, RIL-19, RIL-22, RIL-25, RIL-26, RIL-36, RIL-40, RIL-47, RIL-49, RIL-50, RIL-53, RIL-57, RIL-75, RIL-76, RIL-104, RIL-135, RIL-149, RIL-175, RIL-196, RIL-208, RIL-226, RIL-227, RIL-228, RIL-233, RIL-238, RIL-239, RIL-240, RIL-241, RIL-267, RIL-299, RIL-303, RIL-304, RIL-308, RIL-309, RIL-342, RIL-350, RIL-355, RIL-374, RIL-395, RIL-401, RIL-404, RIL-407, RIL-411, RIL-413, RIL-414, RIL-415, RIL-426, RIL-432, RIL-434, RIL-443, RIL-446, RIL-448, RIL-455, RIL-456, RIL-457, RIL-459, RIL-462, RIL-463, RIL-474, RIL-476
51-75%	4.0	S	166	RIL-10, RIL-16, RIL-17, RIL-18, RIL-21, RIL-28, RIL-31, RIL-32, RIL-33, RIL-34, RIL-37, RIL-42, RIL-43, RIL-44, RIL-45, RIL-46, RIL-51, RIL-52, RIL-63, RIL-64, RIL-68, RIL-73, RIL-82, RIL-84, RIL-88, RIL-91, RIL-92, RIL-103, RIL-105, RIL-111, RIL-115, RIL-116, RIL-117, RIL-119, RIL-121, RIL-125, RIL-127, RIL-137, RIL-140, RIL-141, RIL-143, RIL-146, RIL-151, RIL-155, RIL-158, RIL-159, RIL-169, RIL-170, RIL-171, RIL-173, RIL-174, RIL-178, RIL-179, RIL-180, RIL-184, RIL-186, RIL-188, RIL-189, RIL-194, RIL-197, RIL-201, RIL-204, RIL-209, RIL-212, RIL-213, RIL-214, RIL-215, RIL-216, RIL-225, RIL-231, RIL-236, RIL-237, RIL-242, RIL-243, RIL-244, RIL-247, RIL-249, RIL-250, RIL-251, RIL-253, RIL-255, RIL-256, RIL-266, RIL-270, RIL-273, RIL-275, RIL-279, RIL-280, RIL-281, RIL-282, RIL-284, RIL-285, RIL-288, RIL-289, RIL-298, RIL-301, RIL-306, RIL-307, RIL-313, RIL-315, RIL-318, RIL-319, RIL-321, RIL-323, RIL-328, RIL-329, RIL-330, RIL-333, RIL-338, RIL-339, RIL-340, RIL-343, RIL-344, RIL-345, RIL-346, RIL-347, RIL-352, RIL-356, RIL-357, RIL-358, RIL-364, RIL-365, RIL-366, RIL-371, RIL-372, RIL-373, RIL-375, RIL-377, RIL-378, RIL-379, RIL-380, RIL-381, RIL-382, RIL-383, RIL-387, RIL-388, RIL-389, RIL-391, RIL-397, RIL-400, RIL-402, RIL-405, RIL-417, RIL-422, RIL-423, RIL-424, RIL-425, RIL-430, RIL-435, RIL-438, RIL-440, RIL-441, RIL-444, RIL-450, RIL-451, RIL-454, RIL-458, RIL-464, RIL-465, RIL-467, RIL-470, RIL-477, RIL-481, RIL-483, RIL-484, RIL-485
75-100%	5	HS	58	RIL-2, RIL-6, RIL-27, RIL-54, RIL-60, RIL-61, RIL-74, RIL-101, RIL-112, RIL-114, RIL-122, RIL-123, RIL-134, RIL-139, RIL-147, RIL-156, RIL-160, RIL-162, RIL-168, RIL-181, RIL-182, RIL-187, RIL-190, RIL-193, RIL-211, RIL-217, RIL-221, RIL-222, RIL-254, RIL-311, RIL-337, RIL-341, RIL-367, RIL-369, RIL-370, RIL-392, RIL-393, RIL-394, RIL-396, RIL-399, RIL-403, RIL-408, RIL-412, RIL-416, RIL-419, RIL-420, RIL-421, RIL-437, RIL-445, RIL-447, RIL-449, RIL-461, RIL-468, RIL-469, RIL-482, RIL-487, RIL-488

I=Immune, R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible, HS=highly susceptible

under artificial inoculations out of which four accessions were found to be resistant, three were to be highly susceptible, and the remaining twenty-one were found to be susceptible and none was found to be highly resistant (Asif and Mall 2017, Devi et al., 2018).

### CONCLUSIONS

The disease score of the 338 recombinant inbred lines showed that four lines viz., RIL-210, RIL- 272, RIL-314 and RIL-473 were resistant and forty-seven RILs showed moderately resistant to BLSB. The recombinant inbred lines viz., RIL-6, RIL-26 and RIL-419 were recorded as better genotypes for multiple traits. These RILs would also utilized to improve agronomic performance and disease resistance of maize.

### AUTHOR CONTRIBUTIONS

Conceptualized by Harcharan Singh Dhaliwal and Vikrant Tyagi; Nidhi Devi and Vaishali Sharma performed the experiments. Vikrant Tyagi supervised Nidhi Devi and Vaishali Sharma. Vikrant Tyagi, Imran Sheikh, and Nidhi Devi wrote the original draft. Vikrant Tyagi, Priti Sharma, Praneet Chauhan, and Imran Sheikh reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Received 09 September, 2024; Accepted 22 December, 2024



# Microsatellite Markers: Cross Species Amplification in Myristicaceae

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**Abstract:** Western Ghats of India one of the mega-biodiversity hotspot harbours several important and endemic plant taxa and Myristicaceae is one such example. It is also referred as “nutmeg” family one of the ancient families. Five species of Myristicaceae occur in Western Ghats, among them *Gymnacranthera canarica* and *Myristica fatua* var. *magnifica* are exclusively associated with *Myristica* swamps (India's most threatened ecosystem). Other three species namely *Myristica malabarica* *M. dactyloides* and *Knema attenuate* are non-swampy but are endemic to Western Ghats. Most of these species are listed in RET data book. In this study, an attempt was made to identify microsatellite markers through cross species amplification in Myristicaceae. Totally, 21 microsatellite (SSR) primers that are developed for *Virola sebifera* and *Myristica malabarica* were examined against three different genera. The study indicated possibility of cross species amplification and use of marker for genetic studies in new species. Microsatellite priming sites are more likely to be conserved and thus successful in closely related species (e.g., within subgenera) compared to those that are distantly related (e.g., between genera). Close to 90 percent of species/primer combinations tested within subgenera were successful, a much higher success rate than between genera and geographically distant species.

**Keywords:** Microsatellite, SSR, Cross amplification, Myristicaceae, Swamps

Myristicaceae is one among the ancient angiosperm families, popularly known as nutmeg family (i.e. source of spice such as nutmeg and mace). Myristicaceae are a medium-sized family of angiosperm trees with a wide pan tropical distribution, mostly confined to lowland rainforest habitats (Sauquet 2003) and consist of about 21 genera, with about 520 species (Christenhusz and Byng 2016). The Western Ghats of India, one of the 36 hot spots of Biodiversity in the world (Myers et al., 2000) harbours several species of family Myristicaceae. These Myristicaceae are being used as timber, medicines, food and other purposes (Tambat et al., 2014). Most of the species area are endemic (Ramesh and Pascal 1997) and also listed under endangered a threatened category (FRLHT 2000). Several Myristicaceae members endemic to Western Ghats, show poor regeneration, seed abnormality, discontinuous girth class distribution, etc (Tambat et al., 2007; Tambat et al., 2014). Most geneticists suggested this could be the result of inbreeding and poor genetic structure in endemic species (Savolainen and Kuittinen 2000, Gitzendanner and Soltis 2000).

However, to assess the genetic structure, there are no specific and precise molecular markers for Myristicaceae (Hemmila et al., 2010). Among the available molecular marker, microsatellite (or) simple sequence repeats (SSRs) are the genetic markers of choice for plant and animal systems due to their abundance, co-dominant inheritance, hyper-variability, multi-allelic nature, reproducibility and

transportability between species (Wang et al., 2009) and extensive genome coverage. In forestry, tree species microsatellite (SSR) are found to be power full markers for genetic mapping, diversity analysis, genotyping and SSR markers are capable of detecting large number of alleles, and DNA fingerprinting applications and are used to investigate questions related to effective population size, structure, migration, colonization rates and mating system. The ability of these hyper-variable regions to reveal high allelic diversity and delimit fine genetic structure has resulted in an increase in elaborate population studies (Rossetto et al., 1999, Singh et al., 2014).

The widespread adoption of species-specific SSR (Simple Sequence Repeat) loci in plant research is hindered by their availability for only a limited number of taxa. While many laboratories possess the resources and expertise to conduct SSR - based studies, the characterization of new loci remains a costly and labor-intensive process (Hemmila et al., 2010). An alternative approach involves using microsatellite primers developed for one species to detect polymorphisms in homologous regions of related species. This method significantly reduces resource requirements and broadens the applicability of SSR markers. The success of this heterologous PCR amplification largely depends on the evolutionary proximity between the source and target species. Greater genomic homology often results in higher conservation of SSR-flanking regions, improving primer

transferability (Rossetto 2001). Research has demonstrated that SSR markers can be successfully transferred across species within the same genus and, in some cases, even to species in other genera. This study focuses on examining the cross-species amplification of microsatellite markers originally developed for *Virola sebifera* and *Myristica malabarica* and testing their applicability to other species within the Myristicaceae family of the Western Ghats, India.

### MATERIAL AND METHODS

In this study six species belonging to 3 genera were used. Species such as *Gymnacranthera canarica* (King) Warb. and *Myristica fatua* Houtt. Var *magnifica* (bedd) are known to associate with swampy habitats in Western Ghats. Species namely as *Knema attenuate* (J.Hk and Thw.) Warb) and *Myristica dactyloides* Gaerten, occurs in non-swampy habitat (Chandran et al., 1999, Chandran and Mesta 2001, Tambat et al., 2004, Banik and Bora 2016). However, a lone species known as *Myristica malabarica* Lam. occurs under both swampy and non-swampy conditions (Chandran Mesta 2001) (Table 1). *Myristica fragrance* Menities, Sinclair, (Mollucas island) a domesticated species in India was also used in this study.

**DNA extraction and PCR:** Genomic DNA (g-DNA) was extracted from washed leaf samples using a modified cTAB method (Doyle and Doyle, 1990). The leaf tissue was digested at 65°C for 1 hour in an extraction buffer containing 100 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8), 5 M NaCl, 2% CTAB, 1% PVP-40, and  $\beta$ -mercaptoethanol (2%). The concentration of  $\beta$ -mercaptoethanol was increased to 2% due to the high tannin content in the leaf samples, and SDS was excluded from the buffer to optimize DNA extraction. To

enhance DNA purity, chloroform and isoamyl alcohol (24:1) were added, followed by precipitation of g-DNA with chilled isopropanol. The resulting DNA pellets were washed twice with 70% ethanol and suspended in 10 mM Tris-HCl (pH 8). The extracted DNA was dissolved in TE buffer, and its concentration was measured spectrophotometrically at 260/280 nm. The samples were treated with RNase to remove RNA contamination and analyzed using 0.8% agarose gel electrophoresis. Finally, DNA samples were stored at -20°C for further use.

Microsatellite primer sequences were obtained from published sources (Hemmila et al., 2010; Wei et al., 2013) and synthesized by Bangalore Genie Private Limited, Bangalore. The study utilized 11 primers developed for *Virola sebifera* Aubl. and 10 primers for *Myristica malabarica* Lam (Table 2).

The PCR amplification was carried out in 10 $\mu$ l volume reaction mixture containing 50ng of template DNA, 2.5 mM dNTPs, 4mM each forward and reverse primers, 1.5mM taq buffer and 0.1U taq polymerase. PCR reaction consisted of an initial denaturation at 94°C for 2min followed by 35 cycles of 40 sec denaturation at 94°C, 1min annealing at 50°C and 2min elongation at 72°C. Final elongation was carried out at 72°C for 10min. DNA fragments were separated on 2.5 % agarose gel at 40mA. At each of the PCR amplified microsatellite locus, the frequency of each allele was computed and compared (Wickneswari and Boyle 1999).

### RESULTS AND DISCUSSION

Totally, 21 different primer sets were used (Table 3). Three primers namely Mlr10, M2r6 and M4s14 showed cross amplification with all the five species of Myristicaceae.

**Table 1.** Distribution, habitat and leaf characteristics of the Myristicaceae species of the Western Ghats, India

Species	Habitat	Reference	Roots	Reference	Tree height (in feet)	Leaf characters	Forest types	Distribution	Reference
<i>Gymnacranthera canarica</i> (King) Warb.	Swampy	Tambat et al., 2004	Knee roots	Chandran and Mesta (2001)	Large (Up to 80)	Thin and smooth	Occasional in evergreen forest on windward side of Western Ghats	Endemic to WG	Ramesh and Pascal (1997)
<i>Myristica fatua</i> Houtt. Var. <i>magnifica</i> (Bedd.) Sinclair	Swampy	Gamble (1935)	Prominent stilt root	Chandran and Mesta (2001)	Lofty (Up to 100)	Thick and smooth	In swampy areas of evergreen forest of Western Ghats	Endemic to WG	Ramesh and Pascal (1997)
<i>Myristica malabarica</i> Lam.	Both	Tambat et al., 2004	Stilt root	Chandran and Mesta (2001)	Moderate (Up to 50)	Thin and smooth	Occasional in evergreen forest of Western Ghats	Endemic to WG	Ramesh and Pascal (1997)
<i>Myristica dactyloides</i> Gaertn.	Non-swampy	Tambat et al., 2004	No prominent stilt root	Chandran and Mesta (2001)	Large (Up to 90)	Thick and smooth	Frequent in evergreen forest of Western Ghats	Widespread	
<i>Knema attenuata</i> (J. Hk. & Thw.) Warb.	Non-swampy	Tambat et al., 2004	No prominent stilt root	Chandran and Mesta (2001)	Moderate (Up to 50)	Thin and rough	Frequent in semi-evergreen forest of Western Ghats	Endemic to WG	Ramesh and Pascal (1997)

**Table 2.** Details of the primer sequences, base pairs (bp), annealing temperature and PCR amplification product size range

Primers	Primer sequence	bp	Temp (°C)	PCR product size range (bp)
Mlr6-L	CCTGACAACCTGGCGAAGATGG	21	59.5	141-159
Mlr6-R	TGATTTCAAACCCAGTCAAGG	21		
Mlr10-L	GATCTTGGTGTAACTTCTTCTTC	20	58	145-169
Mlr10-R	CATGCCCCAAAATCACTTC	19		
Mls20-L	GACTGGCCAATATTGATACG	22	63	185-188
Mls20-R	GCAGCAGCAGCAGGAGTTA	19		
Mls75-L	AAGAAAATGGGGCAGACGTT	20	60	155-179
Mls75-R	TGCTGCTAATTCTTCTTTCTCTGAT	25		
M2r6-L	GGTTCACCTGGCCCTTTTGT	20	59.5	141-159
M2r6-R	GATGCACTTAGTAAGGTTTCAAGC	25		
M2r9-L	CTCTCCACATGTCTGAGGAAGCTT	23	59	208-217
M2r9-R	AGGTGATATGGCCCATTTTG	20		
M2r31-L	AGCTTGGGATCAGGTGATATG	21	59	151-159
M2r31-R	CGGCCCAATTGAGCTACTAA	20		
M4S14-L	GTTTACCAGATTGGCACACG	20	63	181-227
M4S14-R	GCACATTGAGTGGACAGCA	19		
M4S73-L	AAGGTCTGGTTTGCTGATGA	20	63	244-268
M4S73-R	AAGGTCTGGTTTGCTGATGA	20		
M4S90-L	CAGCACGGTGACTACAGCAG	20	65	203-239
M4S90-R	TTTTTGGTGTGCTGTCCTTG	20		
M5r33-L	TCAAACAAAACCCACCCATACC	21	62	141-156
M5r33-R	GGTTTAAAAGAGGCCATGATTC	22		
VSE02-L	CGGTAGTCCATTGATTGGCA	20	55	266-296
VSE02-R	GCTGTCATTGTCATCTTCCT	20		
VSE11-L	TATAGATGCCTGCCATTGGA	20	55	237-267
VSE11-R	TCGTGCGAAATTCCTTCTA	20		
VSE30-L	CATGCATGCTGGTCCATA	18	55	159-186
VSE30-R	TTCAGCATATTCTCATGTTCCA	22		
VSE31-L	AACTAGGGCTCTCGCAGCTT	20	55	183-210
VSE31-R	CCAAAGAAGTGCTCCTCAGC	20		
VSE32-L	TGCCCAAGTGGGTTTCTCTA	20	55	197-221
VSE32-R	CCAGTGTCTTCTCTTGCATC	22		
VSE36-L	AGACGGATTGAGGAGAAGCC	20	55	222-243
VSE36-R	CGGAGCACAGGAATGAAATC	20		
VSE38-L	CCATTTGCTCTAAGCAATTCATC	23	55	214-253
VSE38-R	TCACATGCGAATTGTTACAC	21		
VSE42-L	CACCGCTACTGTTTCTGGT	20	55	283-306
VSE42-R	GTGGGATGTGCGATAGAAGC	20		
VSE45-L	TGAAATTTGTTCCCTTCTGAGG	22	55	132-163
VSE45-R	TGATCCATTATTCAGATGAGGC	22		
VSE55-L	GTTGGAGACTGTCTCGGTG	20	55	162-192
VSE55-R	TGCTTAACAGCATGGAATGG	20		

Primers like MIs20, M2r9 and VSE30 exhibited cross amplification probability with four species. Four primers namely Mlr6, MIs75, M2r31 VSE02 showed cross amplification with three species. Remaining primers showed cross amplification less than two species. The two primers Viz., VSE31 and VSE45 showed no cross amplification with Myristicaceae members.

The mean cross amplification across the species observed as 2.04 species per primer. The primers developed for *M. malabarica* (M-series) indicated on an average 2.27 species per primer whereas the primers developed for *Virola sebifera* (VSE series) showed 1.64 species per primer. Number of alleles per locus ranged from 0 to 4 across the species. The primer M2r9 exhibited maximum diversity with an average value 2.00 allele per locus, whereas most other alleles exhibited less than 0.50 alleles per locus with an overall mean of 0.79 alleles per locus.

Cross amplification of *M. Malabarica* primers with the native species *G canarica* showed 45 % success. Similarly with other native species *Knema attenuate*, *Myristica*

*dactyloides* *Myristica fatua* were 18, 64 and 73 percent respectively whereas its was 27 percent with domesticated species *Myristica fragrance*. The cross amplification of *V sebifera* primers with the Western Ghats species namely *G. canarica*, *Myristica fatua* *Knema attenuate* and *Myristica dactyloides* 10, 20, 40 and 50 percent respectively whereas its was 30 percent with domesticated species *Myristica fragrance* (Table 4). The results suggest that within genera cross amplification is high compared to across the genera. Further, Western Ghats species show more cross amplification with native species than with the distant species *V. sebifera*.

These results are in accordance with many previous studies (Zucchi et al., 2002, Yasodha et al., 2005, Williamson et al., 2002, Aldrich, et al., 2003, Wilson et al., 2004, Gutierrez et al., 2005, Magdalena et al., 2007, Barbara et al., 2007, Ravishanker et al., 2011, Wei et al., 2013). They have shown intergeneric transferability of SSR, for instance tall fescue to several grasses (57%), *Litch chinensis* to *Blighia sapida* (58%) and *Setaria italic* to six grass species (74%).

**Table 3.** Primers used and amplification quality, alleles numbers per locus and number of species showed cross amplification

Primer	Gc	Ka	Mm	Mfr	Md	Mfa	Alleles/ locus	# species amplified
Mlr6	*	*	+	*	+	++	1.5	3
Mlr10	+	++	+	*	+	+	1.5	5
MIs20	*	++	+	*	+	+	1	4
MIs75	*	*	+	+	++	—	1	3
M2r6	+	—	+	+	++	++	1.5	5
M2r9	+	—	+	*	+	++	2	4
M2r31	+	—	+	—	—	+	0.5	3
M4s14	+	*	+	+	+	+	1	5
M4s73	*	*	+	*	—	—	0.5	1
M4s90	*	*	+	*	—	—	0.5	1
M5r33	*	*	+	*	*	+	1	2
VSE02	*	+	+	+	—	—	0.5	3
VSE11	*	*	+	+	—	—	0.5	2
VSE30	*	+	+	*	+	+	0.5	4
VSE31	*	*	*	—	—	—	0.5	0
VSE32	+	*	*	*	+	—	1	2
VSE36	*	++	+	*	*	—	0.5	2
VSE38	*	*	*	+	—	+	0.5	2
VSE42	*	*	+	*	—	—	0.5	1
VSE45	*	*	*	*	—	—	0.5	0
VSE55	*	+	*	*	+	—	0.5	2
Mean							0.79	2.04

Amplification quality: ++ very good, + good, \* very poor and — No amplification) Gc-*Gymnacranthera canarica*, Ka-*Knema attenuate*, Mm-*Myristica malabarica*, Mfr-*Myristica fragrance*, Md-*Myristica dactyloides* and Mfa *Myristica fatua*

**Table 4.** Percentage cross amplifications of SSR markers across the species

Species	M-series primer	VSE series primer
<i>Gymnacranthera canarica</i>	45%	10%
<i>Knema attenuate</i>	18 %	40%
<i>Myristica malabarica</i>	100%	50%
<i>Myristica dactyloides</i>	64%	30%
<i>Myristica fatua</i>	73%	20%
<i>Myristica fragrance</i>	27%	30%
Average	55%	30%

M-series – SSR of *Myristica malabarica* VSE series – SSR of *Virola sebifera*

### CONCLUSION

This study demonstrated the utility of *Virola sebifera* and *Myristica malabarica* SSRs markers for the genetic diversity and evolutionary studies of Myristicaceae member of Western Ghats. These results also indicate high levels of cross genera transferability possibility and demonstrated that distantly related species (*Virola sebifera* primers) show less amplification possibility than closely related native species (*Myristica malabarica*). Further, close to 90 percent of species/primer combinations tested within subgenera were successful, a much higher success rate than between genera attempts.

### ACKNOWLEDGMENT

Authors acknowledge the DBT- RGYI, Govt of India, New Delhi and Conservation Leadership Programme (BP Conservation Society, UK) for financial support. Authors also thank the students Anila and Deekhsa and COF, Sirsi.

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# Impact of Organic Amendments on Morphology and Yield Attributes of *Pleurotus ostreatus* Cultivated on Cereal Substrates

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**Abstract:** This study investigated the impact of diverse organic supplements on the yield and morphological characteristics of *Pleurotus ostreatus*, commonly known as Oyster mushrooms. Utilizing locally available supplements, including cereal flour (wheat, rice, maize), pulse flour (soybean, gram, chickpea, lentil, and black gram) and cereal brans (wheat and rice) the experiment assessed their effects on cereal substrate composed of wheat and rice straw at concentrations of 2, 4 and 6 per cent. Among the cereal substrates, rice straw proved to be the most favourable for oyster mushroom cultivation. Soybean flour supplementation on rice straw at 6 per cent concentration gave the highest mean biological yield (195.56) and biological efficiency (97.78%). The highest number of fruit bodies (35.51), pileus diameter (8.05 cm) and stalk length (4.96 cm) were at 6 per cent concentration, while the maximum stalk width (1.95 cm) at 4 per cent soybean flour supplementation. This study highlights the beneficial impact of soybean flour fortification on rice straw in enhancing both morphological characteristics and yield in *Pleurotus ostreatus*.

**Keywords:** *Pleurotus ostreatus*, Supplementation, Organic additives, Biological efficiency

The genus *Pleurotus* belongs to phylum Basidiomycota and is popularly called as "Oyster mushroom". It is also referred to as "*Dhingri*" in India. It is the third largest cultivated mushroom in the world and second largest cultivated mushroom in India (Royse et al 2017). There are about 40 species of *Pleurotus*, which are found in both tropical and temperate climates. Amongst these spp. *Pleurotus eryngii*, *Pleurotus citrinopileatus*, *Pleurotus flabellatus*, *Pleurotus ostreatus*, *Pleurotus djamor var. roseus*, and *Pleurotus florida* have been successfully cultivated using various form of lignocellulosic waste (Bumanlag et al., 2018). *P. ostreatus* is one of many species in this genus, which is widely favoured due to its taste, flavor, high nutritional values and therapeutic qualities (Nowacka-Jechalke et al., 2018, Adebayo et al., 2018). The composition of substrates significantly influences the physicochemical characteristics of mushrooms, playing a crucial role in their productivity and biological efficiency (Belletini et al., 2019). Oyster mushroom grow on variety of substrates, such as paddy straw, maize cobs, vegetable plant leftovers, bagasse, etc. For a long time, wheat straw and sawdust have been the primary lignocellulosic residues used in *Pleurotus* substrate formulations worldwide (Elattar et al., 2019). The cereal straws such as rice, oat, and barley, as well as sugarcane bagasse and grasses also serve as raw materials when these agro-wastes are locally available (Jesus et al., 2023). Mushroom substrates are enriched by adding highly nutritious supplement that provide nutrients to accelerate mycelium growth. Substrates alone may not yield

best result; other ingredients or additives are typically added to them to increase the yield.

Substrate supplementation is a farming technique that involves physically adding nutritious additives to a substrate during the process of removing, the combination of raw ingredients, at spawning or during casing (Pardo-Gimenez et al., 2016). Addition of the supplements with basal substrate has been a common practice to enhance the yield, nutritional and medicinal value by supplying the precise nutrients needed for the growth of the mycelium, which is a component of mushrooms (Naraian et al., 2016). Supplementation substrates play a vital role in promoting growth often by supplying nitrogen through sources including cotton seed meals, wheat bran, and rice bran. Although supplementing the substrate material leads to improved growth but there are limitations involved, high supplementation of substrate may lead to contamination together with reduced yield of the mushrooms. Supplementation led to significant yield increase and even providing up to 34.4 per cent of increment as compared to non-supplementation with deffated pistachio meal for *Pleurotus ostreatus* (Pardo-Giménez et al., 2016).

Mushrooms can utilize nutritional supplements to boost yield by 10 to 20 per cent, sometimes even more, without compromising quality. It is essential to evaluate the types of nutrients, the optimal timing for their application, the cost of the supplements, and the potential economic benefits beforehand (Pardo-Giménez et al., 2018). So when alternative additives are added, mushrooms can grow well

and produce more fruiting bodies. This is typically done by supplementing the substrate with cereal bran such as wheat, soybean, corn, or rice (Josephine 2015, Jesus et al., 2023). *Pleurotus ostreatus* cultivated on the palm waste substrate supplemented with rice bran and wheat bran produced better quality mushrooms (Elkanah et al., 2022). The majority of additives are made of organic resources such as cow dung, palm kernel cake (PKC), groundnut cake (GC), horse gram, cowpea, rice bran, and chicken dung that have proven effective in enhancing mushroom production. Numerous research studies have documented the use of various supplements such as rice bran, wheat bran, corn cobs, corn waste, wheat straw, and banana leaves as substrate supplements for cultivating oyster mushrooms (Muswati et al., 2021, Adebayo et al., 2021, Onyeka et al., 2018, Odunmbaku and Adenipekun 2018).

## MATERIAL AND METHODS

The present investigation was conducted at Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya in Palampur in Centre for Mushroom Research and Training Unit, Department of Plant Pathology.

**Spawn production:** Spawn were prepared by standard procedure (Nidhi 2022).

**Substrate preparation:** Chopped wheat and rice straw, approximately 4-5cm in length, underwent a dual preparation process. Initially, the straw was placed in gunny bags and soaked overnight in fresh water to achieve a moisture level of 70-75 per cent. Following soaking, excess water was drained, and the pre-soaked straw underwent pasteurization by immersion in hot water at 75°C for one and a half hours. After pasteurization, the straw was spread on a plastic sheet and allowed to cool. Moisture content was adjusted to around 65 per cent by assessing it through palm squeezing. Sterilized organic additives including wheat, rice, maize flour, lentil, chickpea, gram, black gram, soybean as well as rice and wheat brans were individually mixed with wheat and rice substrate at three different concentrations based on dry weight before spawning. Subsequently, the spawn was mixed with the substrate, and the mixture was filled into polypropylene bags under aseptic conditions. The bags, tied securely at the top, underwent aeration optimization through 10 to 15 sterile pin-made holes. The spawn run then took place in a growing room with incubation at a temperature of 21-25°C and humidity maintained at 80 per cent.

**Cropping and harvesting:** After the white mycelium had completely covered the substrates, after about 10-12 days the bags were cut open with a sharp, sterilized blade to expose the substrate surface and allow the initiation of pinheads. For the growth of the fruit body, the optimal

environmental conditions of temperature *i.e.* between 20 to 25°C and relative humidity between 70 and 85 per cent were maintained. Ventilation of 2-3 hours per day was given for maintaining CO<sub>2</sub> in the growing room. At regular intervals, observations on the number of days required for pinhead formation were recorded. Bags were watered twice a day. At full maturity, fruiting bodies were harvested. The fruit bodies were harvested before the fruit body curled and its gills were fully developed because over matured fruit bodies become fragile.

### Observation recorded

- i. Number of fruiting body
- ii. Size of fruiting body: The pileus and stalk diameters were recorded by cutting the fruiting body into two parts, the length of the mushroom stalk was recorded.
- iii. Biological yield (BE): Biological yield in g/Kg was calculated by weighing the whole cluster of fruit bodies without removing the straw.
- iv. Economic yield (EY): Economic yield in g/Kg was calculated by weighing all the fruiting bodies after removing the straw.
- v. Biological efficiency: Biological efficiency of total harvests was calculated by using the formula

$$\text{Biological Efficiency (\%)} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

**Statistical analysis:** The data were analysed by using OPSTAT software (Steel et al 1997).

## RESULTS AND DISCUSSION

### Effect of Cereal Flour Supplementation

**Effect on yield parameters of *Pleurotus ostreatus* :** The significantly mean maximum biological yield (187.24 g/kg), economical yield (170.79 g/kg) and biological efficiency (92.70%) was in rice flour supplementation (Table 1, Plate 1) followed by wheat flour. Irrespective of the additives, the significantly maximum biological yield (192.54g/kg), economical yield (178.38g/kg) and biological efficiency (96.41%) was recorded when wheat substrate supplemented with rice flour at 6 per cent concentration. Significantly mean minimum (140.32g/kg) biological yield, economical yield (130.53g/kg) and biological efficiency (70.12%) was observed in control. Rice flour was the effective additive in wheat substrate for *Pleurotus ostreatus*. Kumar et al (2020) reported *Pleurotus sapidus* and *Pleurotus flabellatus* gave highest yield when wheat straw supplemented with rice flour @2.5 per cent.

**Effect on morphological characters of *Pleurotus ostreatus*:** Significantly mean maximum number of fruit bodies (28.55), pileus diameter (7.53 cm) and stalk width (1.57 cm) in rice flour supplementation (Table 2) followed by

wheat flour whereas, mean highest stalk length was reported in wheat flour additive (4.05 cm) followed by rice and maize flour. Mean minimum number of fruit bodies (15.32), pileus diameter (6.50 cm), stalk length (3.18) and stalk width (1.34 cm) was found in control. Irrespective of the additives, significantly maximum number of fruit bodies (30.73) was observed at 6 per cent concentration and stalk width (1.92 cm) at 2 per cent concentration of rice flour additive whereas, pileus diameter (7.84 cm) and stalk length (4.56) in wheat flour at 6 per cent. Soam et al. (2018) and Kumar et al. (2020) also reported maximum stalk length and width of *P. ostreatus* after adding rice flour as compare to control.

#### Effect of Pulse Flour Supplementation

##### Effect on yield parameters of *Pleurotus ostreatus*:

Among five pulse flour additives, significantly mean maximum biological yield (181.09 g/kg), economical yield (162.99 g/kg) and biological efficiency (90.76%) was in soybean flour (Table 3) followed by chickpea flour whereas, mean minimum biological yield (140.32 g/kg), economical

yield (130.53 g/kg) and biological efficiency (70.12%) was in control. Irrespective of additives, significant maximum biological yield (185.23 g/kg), economical yield (166.92g/kg) and biological efficiency (92.50%) was at 6 per cent concentration after adding soybean flour and minimum biological yield (140.32 g/kg), economical yield (130.53g/kg) and biological efficiency (70.12%) in control. The results emphasize the positive impact of pulse flour fortification, with soybean flour demonstrating superior effects on the yield parameters of *Pleurotus ostreatus*. Singh et al. (2017) observed that supplementing wheat straw with various pulse flours resulted in maximum yield with pigeon pea flour followed by soybean flour, while the control exhibited the minimum yield.

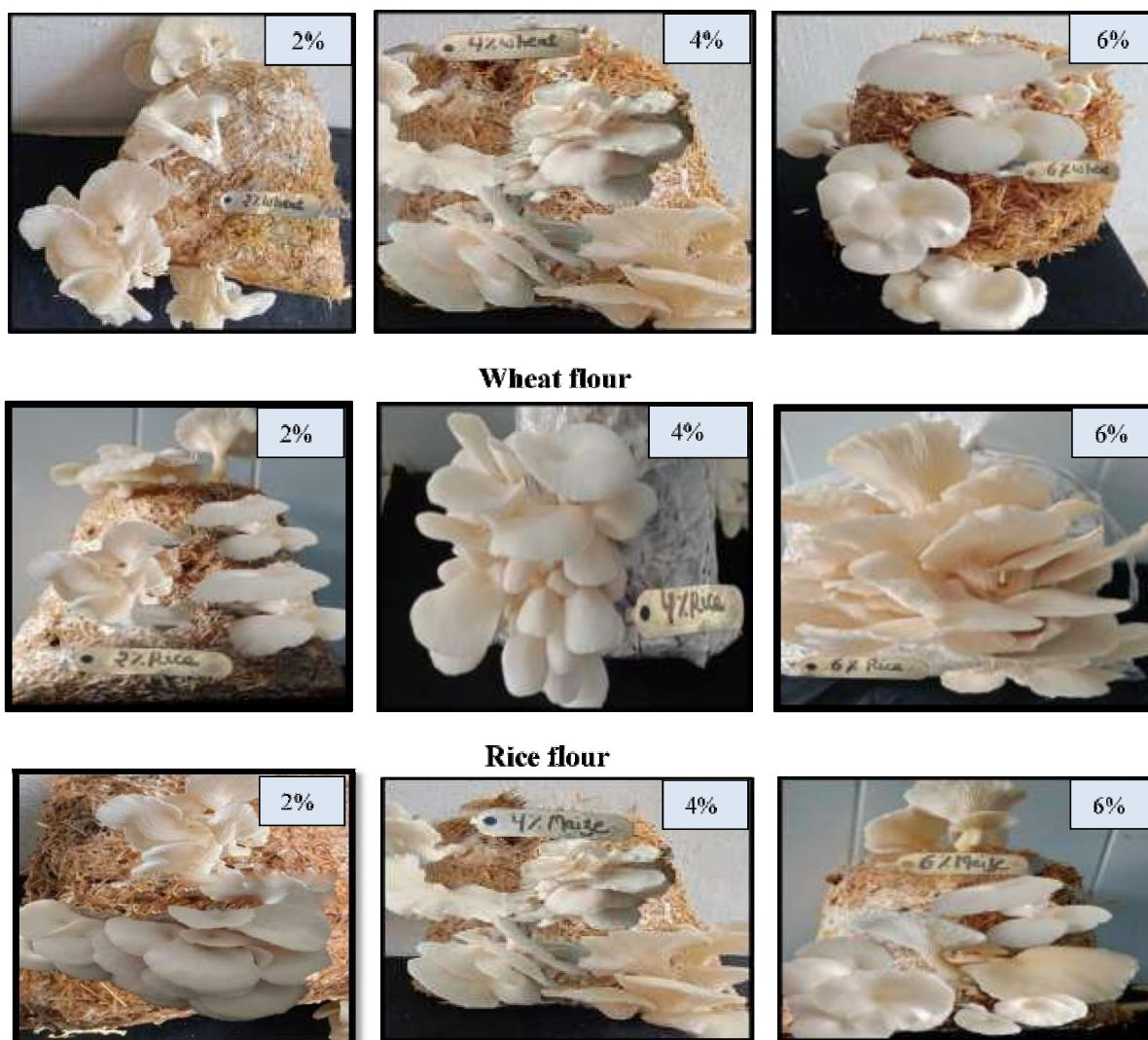
Effect on morphological characters of *Pleurotus ostreatus*: Significantly mean maximum number of fruit bodies (24.76), stalk length (4.12 cm) and stalk width (1.63 cm) was observed in soybean flour additive followed by lentil flour whereas, mean maximum pileus diameter in chickpea

**Table 1.** Effect of cereal flour supplementation on the yield parameters of *Pleurotus ostreatus* on wheat straw

Flour	Biological yield (g/kg) concentration (%)			Mean	Economical yield (g/kg) concentration (%)			Mean	Biological efficiency (%) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6	
Wheat	171.33	180.52	174.75	175.53	153.30	164.49	159.73	159.17	85.51	89.57	89.66	88.25
Rice	180.62	188.56	192.54	187.24	168.35	176.62	178.38	170.79	91.28	94.41	96.41	92.70
Maize	168.25	179.69	176.80	174.92	152.12	160.45	158.26	156.94	84.58	88.34	87.32	86.75
Control	140.32	140.32	140.32	140.32	130.53	130.53	130.53	130.53	70.12	70.12	70.12	70.12
Mean	165.13	172.27	171.10		153.08	155.52	154.48		83.35	85.83	85.60	
CD (p=0.05)												
Supplements (A)			5.85		Supplements (A)		3.41		Supplements (A)		3.63	
Supplements (B)			5.04		Supplements (B)		NS		Supplements (B)		NS	
Interaction (A×B)			NS		Interaction (A×B)		5.91		Interaction (A×B)		NS	

**Table 2.** Effect of cereal flour supplementation on the morphological characters of *Pleurotus ostreatus* on wheat straw

Flour	Fruit bodies (no.) concentration (%)			Mean	Pileus diameter (cm) concentration (%)			Mean	Stalk length (cm) concentration (%)			Mean	Stalk width (cm) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6		2	4	6	
Wheat	24.24	27.37	30.48	27.70	6.92	7.10	7.84	7.24	3.59	4.00	4.56	4.05	1.12	1.45	1.48	1.35
Rice	26.54	28.38	30.73	28.55	7.25	7.81	7.52	7.53	3.42	3.79	4.20	3.80	1.92	1.37	1.42	1.57
Maize	22.20	23.56	25.42	23.73	6.82	7.50	7.50	7.17	3.29	3.56	3.92	3.59	1.00	1.27	1.45	1.24
Control	15.32	15.32	15.32	15.32	6.50	6.50	6.50	6.50	3.18	3.18	3.18	3.18	1.00	1.00	1.00	1.00
Mean	22.33	23.66	25.49		6.87	7.12	7.34		3.37	3.63	3.97		1.26	1.27	1.34	
CD (p=0.05)																
Supplements (A)		0.63		Supplements (A)		0.16		Supplements (A)		0.09		Supplements (A)		0.03ff		
Supplements (B)		0.55		Supplements (B)		0.13		Supplements (B)		0.08		Supplements (B)		0.02		
Interaction (A×B)		1.10		Interaction (A×B)		0.27		Interaction (A×B)		0.16		Interaction (A×B)		0.05		



**Plate 1.** Cereal flour supplementation on yield of *Pleurotus ostreatus* on wheat substrate

**Table 3.** Effect of pulse flour supplementation on the yield parameters of *Pleurotus ostreatus* on wheat straw

Flour	Biological yield (g/kg) concentration (%)			Mean	Economical yield (g/kg) concentration (%)			Mean	Biological efficiency (%) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6	
Soybean	177.49	180.54	185.23	181.09	159.58	162.46	166.92	162.99	88.50	91.27	92.50	90.76
Chickpea	162.56	169.24	160.15	163.99	152.42	154.17	150.23	152.27	81.28	84.62	80.07	81.99
Lentil	142.52	148.56	150.24	147.11	129.21	130.46	129.62	129.76	71.26	74.28	75.12	73.56
Gran	158.27	160.52	163.75	160.85	142.23	144.56	146.23	144.34	79.85	80.26	81.52	80.54
Black gram	155.62	152.69	161.54	156.62	144.23	137.52	149.73	143.83	77.21	76.34	80.52	78.02
Control	140.32	140.32	140.32	140.32	130.53	130.53	130.53	130.53	70.12	70.12	70.12	70.12
Mean	156.13	158.65	160.21		143.03	143.28	145.54		78.04	79.48	79.98	
C.D.(p=0.05)												
Supplements (A)		4.03		Supplements (A)		3.78		Supplements (A)		1.99		
Supplements (B)		2.85		Supplements (B)		NS		Supplements (B)		1.41		
Interaction (A×B)		NS		Interaction (A×B)		NS		Interaction (A×B)		NS		

flour supplementation (7.07 cm) followed by lentil and soybean flour additive (Table 4). However, mean minimum fruit bodies (15.32), pileus diameter (6.50 cm), stalk length (3.18 cm) and stalk width (1.00 cm) was in control. Irrespective of different additives, significantly maximum number of fruit bodies (26.15) and stalk width (1.83 cm) in soybean flour at 6 per cent concentration and pileus diameter (7.35 cm) at highest concentration of lentil flour, stalk length (4.35 cm) at 4 per cent soybean flour whereas, minimum fruit bodies (15.30) in chickpea flour and pileus diameter (6.42 cm) in gram flour at 2 per cent concentration, stalk length (3.18 cm) and stalk width (1.00 cm) was in control. Singh et al (2017) observed that increased supplement dose of soybean improves the morphological characters of *Pleurotus ostreatus*.

#### Effect of Cereal Bran Supplementation

**Effect on yield parameters of *Pleurotus ostreatus*:** The

significantly mean maximum biological yield (165.61g/kg), economical yield (149.51g/kg) and biological efficiency (82.77%) was observed in wheat bran supplementation followed by rice bran whereas, mean minimum biological yield (140.32g), economical yield (130.53g) and biological efficiency (70.12%) in control (Table 5). Irrespective of brans, maximum biological yield (167.19 g/kg), economical yield (156.52 g/kg), biological efficiency (83.59%) was observed at 4 per cent concentration of wheat bran and minimum biological yield (140.32g/kg), economical yield (130.53g/kg), biological efficiency (70.12%) in control. Rout et al (2016) also reported superior yield in wheat straw supplemented with rice bran followed by wheat bran.

**Effect on morphological characters of *Pleurotus ostreatus* :** Significantly mean maximum number of fruit bodies (22.35), maximum pileus diameter (6.75 cm), mean stalk width (1.32 cm) was observed in wheat bran as additive

**Table 4.** Effect of pulse flour supplementation on the morphological characters of *Pleurotus ostreatus* on wheat straw

Flour	Fruit bodies (no.) concentration (%)			Mean	Pileus diameter (cm) concentration (%)			Mean	Stalk length (cm) concentration (%)			Mean	Stalk width (cm) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6		2	4	6	
Soybean	22.54	25.60	26.15	24.76	6.56	6.72	7.00	6.76	3.82	4.35	4.20	4.12	1.42	1.64	1.83	1.63
Chickpea	15.30	17.26	18.39	16.98	6.82	7.12	7.29	7.07	3.62	3.97	4.32	3.97	1.36	1.28	1.54	1.39
Lentil	22.47	23.85	20.19	22.17	6.45	6.92	7.35	6.91	3.42	3.67	3.92	3.67	1.14	1.26	1.35	1.25
Gram	18.64	15.92	20.75	18.44	6.42	6.71	7.00	6.71	3.52	3.74	3.56	3.61	1.16	1.27	1.20	1.21
Black gram	18.53	17.58	15.37	17.16	6.53	6.64	6.82	6.66	3.45	3.52	3.93	3.66	1.12	1.42	1.21	1.25
Control	15.32	15.32	15.32	15.32	6.50	6.50	6.50	6.50	3.18	3.18	3.18	3.18	1.00	1.00	1.00	1.00
Mean	18.80	19.26	19.36		6.55	6.77	6.99		3.50	3.94	4.27		1.20	1.31	1.35	
CD (p=0.05)																
Supplements (A)	0.43				Supplements (A)			0.14	Supplements (A)			0.11	Supplements (A)			0.04
Supplements (B)	0.30				Supplements (B)			0.10	Supplements (B)			0.08	Supplements (B)			0.03
Interaction (A×B)	0.74				Interaction (A×B)			0.24	Interaction (A×B)			0.19	Interaction (A×B)			0.06

**Table 5.** Effect of cereal bran supplementation at different concentrations on the yield parameters of *Pleurotus ostreatus* on wheat straw

Flour	Biological yield (g/kg) concentration (%)			Mean	Economical yield (g/kg) concentration (%)			Mean	Biological efficiency (%) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6	
Wheat	165.43	167.19	164.21	165.61	148.27	156.52	149.73	149.51	82.62	83.59	82.10	82.77
Rice	158.24	162.17	164.34	161.58	142.56	140.17	149.21	145.98	79.12	81.56	82.67	81.12
Control	140.32	140.32	140.32	140.32	130.53	130.53	130.53	130.53	70.12	70.12	70.12	70.12
Mean	154.66	150.56	156.29		140.45	142.41	143.16		77.29	78.42	78.30	
CD (p=0.05)												
Supplements (A)	5.02				Supplements (A)			3.56	Supplements (A)			2.08
Supplements (B)	NS				Supplements (B)			NS	Supplements (B)			NS
Interaction (A×B)	NS				Interaction (A×B)			6.16	Interaction (A×B)			NS

followed by rice bran supplementation whereas, mean maximum stalk length in rice bran (3.72 cm) followed by wheat bran (Table 6). However, significantly mean minimum number of fruit bodies (15.32), pileus diameter (6.50 cm), stalk length (3.18 cm) and stalk width (1.00 cm) was in control. Irrespective of the bran supplementation, maximum number of fruit bodies (23.46) was observed at 4 per cent, pileus diameter (6.95 cm) at 6 per cent, stalk width (1.54 cm) at 6 per cent of wheat bran and stalk length (3.80 cm) at 2 per cent of rice bran supplementation whereas, the minimum fruit bodies (15.32), pileus diameter (6.50 cm), stalk length (3.18 cm) and stalk width (1.00 cm) was in control. This study emphasizes the positive influence of cereal bran fortification on morphological characters in *Pleurotus ostreatus*. Rout et al. (2016) reported improvements in morphological character when wheat straw supplemented with rice bran followed by wheat bran.

#### Effect of different concentrations of organic supplementation on yield parameters and morphological characters of *Pleurotus ostreatus* on rice straw

#### Effect of Cereal Flour Supplementation

**Effect on yield parameters of *Pleurotus ostreatus*:** The significantly mean maximum biological yield (195.35 g/kg), economical yield (184.31 g/kg) and biological efficiency (97.67%) was reported in wheat flour supplementation followed by rice flour (Table 7). Irrespective of the cereal flour amendments, significantly maximum (198.62 g/kg) biological yield, economical yield (187.92 g/kg) and biological efficiency (99.31%) was recorded when rice substrate supplemented with wheat flour at 6 per cent concentration followed by 4 per cent concentration. Thereafter, significantly mean minimum (165.42g/kg) biological yield, economical yield (152.46 g/kg) and biological efficiency (81.21%) was in control. All treatments demonstrated significant superiority over the control, emphasizing the positive impact of cereal flour supplementation on *Pleurotus ostreatus* cultivation on rice straw.

**Effect on morphological characters of *Pleurotus ostreatus*:** Significantly mean maximum number of fruit bodies (26.81) was observed in maize flour supplementation maximum pileus diameter (7.92 cm) and stalk width (1.48

**Table 6.** Effect of cereal bran supplementation on the morphological characters of *Pleurotus ostreatus* on wheat straw

Flour	Fruit bodies (no.) concentration (%)			Mean	Pileus diameter (cm) concentration (%)			Mean	Stalk length (cm) concentration (%)			Mean	Stalk width (cm) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6		2	4	6	
	Wheat	20.86	23.46		22.72	22.35	6.58		6.71	6.95	6.75		3.56	3.78	3.40	
Rice	18.00	22.56	20.53	20.37	6.60	6.73	6.82	6.72	3.80	3.72	3.64	3.72	1.24	1.12	1.46	1.27
Control	15.32	15.32	15.32	15.32	6.50	6.50	6.50	6.50	3.18	3.18	3.18	3.18	1.00	1.00	1.00	1.00
Mean	18.06	20.45	19.52		6.56	6.65	6.75		3.51	3.56	3.41		1.14	1.12	1.33	
CD (p=0.05)																
Supplements (A)		0.55		Supplements (A)		0.15		Supplements (A)		0.08		Supplements (A)		0.02		0.02
Supplements (B)		0.55		Supplements (B)		0.15		Supplements (B)		0.08		Supplements (B)		0.02		0.02
Interaction (A×B)		0.94		Interaction (A×B)		NS		Interaction (A×B)		0.15		Interaction (A×B)		0.04		0.04

**Table 7.** Effect of cereal flour supplementation on the yield parameters of *Pleurotus ostreatus* on rice straw

Flour	Biological yield (g/kg) concentration (%)			Mean	Economical yield (g/kg) concentration (%)			Mean	Biological efficiency (%) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6	
	Wheat	192.41	195.00		198.62	195.35	180.57		184.43	187.92	184.31	
Rice	180.52	184.71	187.93	184.39	170.53	173.27	175.42	173.07	90.26	91.35	93.96	91.86
Maize	178.31	180.74	184.23	181.09	164.48	167.36	172.54	168.13	89.15	90.37	92.11	90.54
Control	165.42	165.42	165.42	165.42	152.46	152.46	152.46	152.46	81.21	81.21	81.21	81.21
Mean	179.17	181.47	184.05		167.01	169.38	172.09		89.20	90.11	91.65	
CD (p=0.05)												
Supplements (A)			4.09	Supplements (A)			3.94	Supplements (A)			1.92	
Supplements (B)			3.54	Supplements (B)			3.47	Supplements (B)			1.66	
Interaction (A×B)			NS	Interaction (A×B)			NS	Interaction (A×B)			NS	

cm) in wheat flour as additive whereas, stalk length (3.66 cm) in wheat and rice flour supplementation (Table 8). Significantly mean minimum number of fruit bodies (17.54), pileus diameter (5.83 cm), stalk length (3.20 cm) and stalk width (1.00 cm) was recorded in control. Irrespective of additives, maximum number of fruit bodies (32.74), pileus diameter (8.05 cm), stalk length (3.81 cm) and stalk width (1.62 cm) was recorded at 6 per cent concentration of wheat flour additive however, the minimum fruit bodies (17.54), pileus diameter (5.83 cm) and stalk width (1.00 cm) was observed in control, while, stalk length (3.15 cm) at 2 per cent concentration of maize flour. The 6 per cent concentration of the supplements led to improvements in morphological characters. Kumar et al (2020) also reported *Pleurotus sapidus* and *Pleurotus flabellatus* maximum number of sporophore@2.5 per cent and maximum width @ 2.0 per cent of rice flour supplementation followed by wheat flour.

### Effect of Pulse Flour Supplementation

#### Effect on yield parameters of *Pleurotus ostreatus*:

Significantly mean maximum biological yield (195.56 g/kg), economical yield (171.87 g/kg) and biological efficiency (97.78%) was in soybean flour addition followed by chickpea flour (Table 9). Irrespective of the pulse flour, maximum biological yield (198.80 g/kg), economical yield (187.80 g/kg) and biological efficiency (99.40%) was recorded when rice substrate supplemented with soybean flour supplementation at 6 per cent concentration followed by 4 per cent concentration of same additive. Whereas, mean minimum biological yield (165.42g/kg), economical yield (152.46 g/kg) and biological efficiency (81.21%) was in control. Bhadana (2014) also reported Pigeon pea flour supplements increased the yield of *P. florida* and *P. djamor*.

### Effect of Pulse Flour Supplementation

#### Effect on morphological characters of *Pleurotus*

**Table 8.** Effect of cereal flour supplementation at different concentrations on the morphological characters of *Pleurotus ostreatus* on rice straw

Flour	Fruit bodies (no.) concentration (%)			Mean	Pileus diameter (cm) concentration (%)			Mean	Stalk length (cm) concentration (%)			Mean	Stalk width (cm) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6		2	4	6	
Wheat	25.79	30.53	32.74	29.69	7.80	7.92	8.05	7.92	3.46	3.72	3.81	3.66	1.25	1.58	1.62	1.48
Rice	25.76	29.48	26.20	27.14	6.82	6.74	6.90	6.82	3.52	3.65	3.80	3.66	1.25	1.37	1.47	1.35
Maize	26.42	25.68	28.44	26.81	7.12	7.46	7.62	7.40	3.15	3.26	3.59	3.33	1.20	1.37	1.56	1.38
Control	17.54	17.54	17.54	17.54	5.83	5.83	5.83	5.83	3.20	3.20	3.20	3.20	1.00	1.00	1.00	1.00
Mean	23.88	25.80	26.23		6.89	6.99	7.10		3.33	3.46	3.60		1.18	1.33	1.42	
CD (p=0.05)																
Supplements (A)				0.66	Supplements (A)			0.17	Supplements (A)			0.06	Supplements (A)			0.03
Supplements (B)				0.57	Supplements (B)			0.14	Supplements (B)			0.06	Supplements (B)			0.02
Interaction (A×B)				1.14	Interaction (A×B)			NS	Interaction (A×B)			0.11	Interaction (A×B)			0.05

**Table 9.** Effect of pulse flour supplementation on the yield parameters of *Pleurotus ostreatus* on rice straw

Flour	Biological yield (g/kg) concentration (%)			Mean	Economical yield (g/kg) concentration (%)			Mean	Biological efficiency (%) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6	
Soybean	192.37	195.52	198.80	195.56	180.42	184.39	187.80	171.87	96.68	97.26	99.40	97.78
Chickpea	174.35	178.72	182.21	178.43	163.27	167.45	180.73	170.48	87.17	89.53	91.10	89.27
Lentil	168.52	172.39	170.16	170.36	156.42	161.78	160.49	159.56	84.26	86.19	85.53	85.32
Gran	172.48	180.74	176.31	176.51	161.42	169.37	164.45	165.08	86.25	90.37	88.15	88.26
Black gram	164.31	168.46	160.40	164.39	154.38	156.72	149.47	153.52	82.86	84.23	80.20	82.43
Control	165.42	165.42	165.42	165.42	152.46	152.46	152.46	152.46	81.21	81.21	81.21	81.21
CD (p=0.05)												
Supplements (A)				3.52	Supplements (A)			3.56	Supplements (A)			2.45
Supplements (B)				2.49	Supplements (B)			2.52	Supplements (B)			NS
Interaction (A×B)				NS	Interaction (A×B)			6.17	Interaction (A×B)			NS



**ostreatus:** Significantly mean maximum number of fruit bodies (32.08) and maximum pileus diameter (7.92 cm) in soybean flour supplementation, whereas, stalk length (4.67 cm) and stalk width (1.61 cm) in addition of chickpea flour (Table 10). Significantly mean minimum number of fruit bodies (17.54), pileus diameter (5.83 cm), stalk length (3.20 cm) and stalk width (1.00 cm) was in control. Irrespective of supplementation, maximum number of fruit bodies (35.51) and pileus diameter (8.05 cm) in soybean flour, stalk length (4.96 cm) in chickpea at 6 per cent concentration and stalk width (1.95 cm) at 4 per cent concentration of soybean flour. Bhadana (2014) also reported pigeon pea flour increased number of sporophores of *P. florida* and *P. djamor*. Maximum sporophores yield and average numbers of sporophores were in *P. flabellatus* and *P. djamor* respectively in pigeon pea flour.

### Effect of Cereal Bran Supplementation

**Effect on yield parameters *Pleurotus ostreatus*:** Among

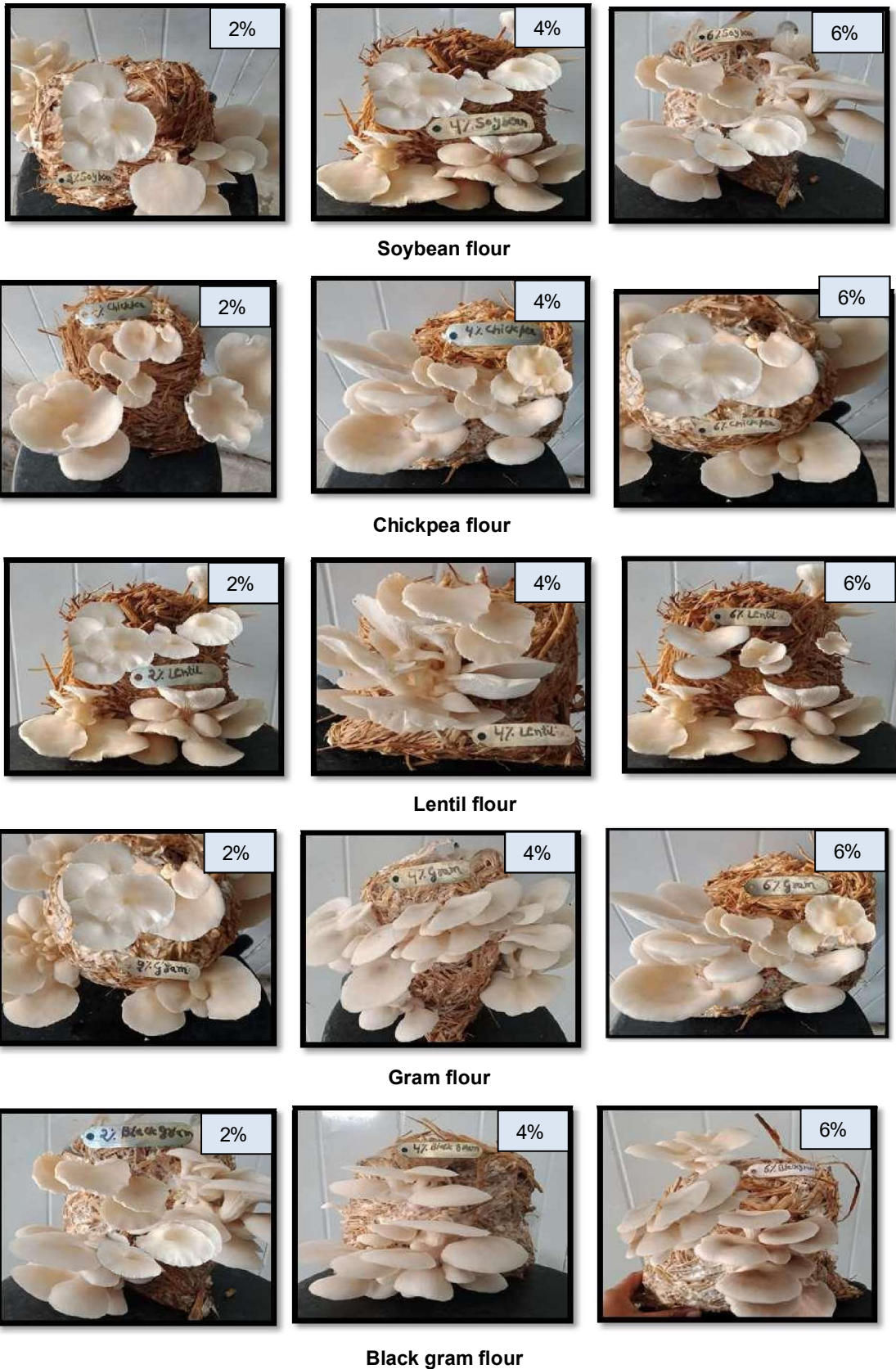
two cereal bran, significantly mean maximum biological yield (184.66 g/kg), economical yield (173.43 g/kg) and biological efficiency (94.46%) was observed in rice substrate with rice bran supplementation followed by wheat bran and minimum in control (Table 11). Whereas, irrespective of bran additives, maximum (188.38 g/kg) biological yield, economical yield (176.29 g/kg) and biological efficiency (94.19 %) was recorded when substrate supplemented with rice bran supplementation at 6 per cent concentration followed by 4 per cent concentration of same additive and minimum (165.42 g/kg) biological yield, economical yield (152.46 g/kg) and biological efficiency (81.21%) was recorded in control. Increased in the bran concentration increased the yield in rice bran supplementation but in wheat bran the maximum yield was observed at 4 per cent concentration. Buendia et al. (2016) also reported that addition of rice bran supplements to the substrate significantly improves biological efficiency of mushrooms. Tripathy and Nayak (2022) showed the highest

**Table 10.** Effect of pulse flour supplementation on the morphological characters of *Pleurotus ostreatus* on rice straw

Flour	Fruit bodies (no.) concentration (%)			Mean	Pileus diameter (cm) concentration (%)			Mean	Stalk length (cm) concentration (%)			Mean	Stalk width (cm) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6		2	4	6	
Soybean	28.26	32.49	35.51	32.08	7.80	7.92	8.05	7.92	3.57	3.72	4.32	3.87	1.82	1.95	1.20	1.59
Chickpea	20.52	23.69	24.16	22.79	7.24	7.46	7.51	7.40	4.45	4.60	4.96	4.67	1.32	1.64	1.86	1.61
Lentil	24.62	25.47	26.56	25.55	6.25	6.59	6.76	6.53	3.67	3.74	4.16	3.86	1.24	1.46	1.50	1.40
Gram	18.29	22.62	20.38	20.43	6.67	6.82	7.41	6.97	3.49	3.83	4.00	3.77	1.20	1.28	1.30	1.26
Black gram	22.64	23.29	25.61	23.85	6.74	6.83	6.90	6.82	3.62	3.75	3.94	3.77	1.26	1.47	1.49	1.41
Control	17.54	17.54	17.54	17.54	5.83	5.83	5.83	5.83	3.20	3.20	3.20	3.20	1.00	1.00	1.00	1.00
Mean	21.31	23.52	29.13		6.75	6.89	7.04		3.67	3.81	4.10		1.31	1.47	1.36	
CD (p=0.05)																
Supplements (A)				0.61	Supplements (A)			0.15	Supplements (A)			0.09	Supplements (A)			0.03
Supplements (B)				0.43	Supplements (B)			0.10	Supplements (B)			0.06	Supplements (B)			0.02
Interaction (A×B)				1.05	Interaction (A×B)			0.26	Interaction (A×B)			0.16	Interaction (A×B)			0.05

**Table 11.** Effect of cereal bran supplementation at different concentrations on the yield parameters of *Pleurotus ostreatus* on rice straw

Flour	Biological yield (g/kg) concentration (%)			Mean	Economical yield (g/kg) concentration (%)			Mean	Biological efficiency (%) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6	
Wheat	170.52	178.39	172.64	173.85	150.27	166.54	161.80	159.54	85.26	89.54	86.32	87.04
Rice	180.17	185.42	188.38	184.66	168.52	175.47	176.29	173.43	90.47	92.71	94.19	94.46
Control	165.42	165.42	165.42	165.42	152.46	152.46	152.46	152.46	81.21	81.21	81.21	81.21
Mean	172.04	176.41	175.48		157.08	164.82	163.52		85.65	87.82	87.24	
CD (p=0.05)												
Supplements (A)				3.33	Supplements (A)			3.71	Supplements (A)			2.18
Supplements (B)				3.33	Supplements (B)			3.71	Supplements (B)			NS
Interaction (A×B)				NS	Interaction (A×B)			6.42	Interaction (A×B)			NS



**Plate 2.** Effect of pulse flour supplementation on yield parameters of *Pleurotus ostreatus* on rice substrate

**Table 12.** Effect of cereal bran supplementation on the morphological characters of *Pleurotus ostreatus* on rice straw

Flour	Fruit bodies (no.) concentration (%)			Mean	Pileus diameter (cm) concentration (%)			Mean	Stalk length (cm) concentration (%)			Mean	Stalk width (cm) concentration (%)			Mean
	2	4	6		2	4	6		2	4	6		2	4	6	
Wheat	18.25	20.64	20.72	19.87	6.85	6.95	7.21	7.00	3.64	3.92	4.20	3.92	1.27	1.36	1.39	1.34
Rice	22.38	24.56	27.29	24.74	6.35	6.39	6.40	6.38	3.29	3.57	3.63	3.50	1.56	1.59	1.60	1.58
Control	17.54	17.54	17.54	17.54	5.83	5.83	5.83	5.83	3.20	3.20	3.20	3.20	1.00	1.00	1.00	1.00
Mean	19.39	20.91	21.85		6.34	6.39	6.48		3.38	3.56	3.68		1.28	1.32	1.33	
CD (p=0.05)																
Supplements (A)				0.58	Supplements (A)			0.13	Supplements (A)			0.10	Supplements (A)			0.03
Supplements (B)				0.58	Supplements (B)			NS	Supplements (B)			0.10	Supplements (B)			0.02
Interaction (A×B)				1.01	Interaction (A×B)			NS	Interaction (A×B)			0.17	Interaction (A×B)			0.04

biological yield of *Pleurotus ostreatus* was also obtained from paddy straw and wheat bran (90:10) followed by paddy straw and wheat bran (95:5).

**Effect on morphological characters of *Pleurotus ostreatus*:** The significantly maximum number of fruit bodies (24.74) and stalk width (1.58 cm) was observed in rice bran, whereas, maximum pileus diameter (7.00 cm) and stalk length (3.92 cm) in wheat bran supplementation as compared to control (Table 12). Irrespective of bran supplementation, maximum number of fruit bodies (27.29) and stalk width (1.60 cm) in rice flour supplementation at 6 per cent, while, pileus diameter (7.21 cm) and stalk length (4.20 cm) in wheat bran at 6 per cent concentration. Minimum number of fruit bodies (17.54), pileus diameter (5.83 cm), stalk length (3.20 cm) and stalk width (1.00 cm) was in control. Tripathy and Nayak (2022) who reported that paddy straw with wheat bran (90:10) supplementation gave maximum number of fruiting bodies of *Pleurotus* sp.

### CONCLUSION

Bio fortification of cereal substrate with organic additives had a positive effect on morphological parameters and biological efficiency of *P. ostreatus*. Rice straw among cereal substrate performed better and gave better yield and improved morphological characters. Addition of supplements in rice straw with wheat flour (cereal), soybean flour (pulse) and rice bran (cereal bran) at 6 per cent concentration enhanced the yield and improved morphological parameters. It was concluded that increasing in the supplementation dose helped to increase every aspect of yield and morphological characteristics. Supplementation had a positive impact on the cultivation of *Pleurotus ostreatus*.

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Received 05 October, 2024; Accepted 24 January, 2025



# Abundance of Insect Pollinators on *Calotropis procera* in Buxar district of Bihar

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**Abstract:** This comprehensive study sheds light on the diverse insect forager species associated with *Calotropis procera*, revealing 21 (twenty-one) species, including two unidentified ones, distributed across five orders. The population abundance assessment over a six-month period uncovered dynamic trends, with March recording the highest mean population of pollinators. Ants emerged as consistent visitors, with *Apis dorsata* dominating in December. Diversity indices analysis indicated rich pollinator diversity in areas, with the Simpson's index reflecting values from 0.942 to 0.955, suggesting a robust and diverse pollinator community. The highest species richness was in March at location having latitude- 84°9'25", longitude- 25°31'4". The study advocates for the conservation of pollinators with weeds as a potential strategy to address threats to biodiversity, aligning with previous research emphasizing the positive role of weeds in enhancing floral diversity and supporting insect populations.

**Keywords:** *Calotropis procera*, Pollinators, Relative abundance, Diversity index and Bihar

*Calotropis* includes *Calotropis procera* and *C. gigantea* are perennial wild herb widely distributed in tropical and subtropical areas such as India, Africa, Egypt, Pakistan, Iran, Arabic islands and Australia. *Calotropis* is a staple in arid and marginal areas and is essential to the health of the ecosystem. Its robust root systems contribute to soil conservation, preventing erosion and aiding in the adaptation to challenging environmental conditions, thereby combating desertification (Nascimento et al., 2015). This makes *calotropis* instrumental in preserving the integrity of ecosystems in regions facing arid challenges. The plant's importance extends to traditional medicine and crafts, enriching the lives of local communities. Various parts of *calotropis*, such as roots, leaves, and latex, have been used in traditional medicinal practices for their anti-inflammatory, anti-cancer, and anti-microbial properties (Wani et al., 2017). Additionally, the fibres derived from *calotropis* stems are employed in crafting ropes and traditional products, contributing to local economies (Verma et al., 2018).

The *calotropis* flower acts as a nectar source for flower visitors and having some demonstrated co-evolutionary relationships with each other (Salau and Nasiru 2015). The flower visitors pertain to the *C. Prospera* plant, mainly from the Hymenoptera order, encompassing carpenter bees, honeybees, ants, and wasps. Additionally, there are a few species from the Diptera and Lepidoptera orders (Zafar et al., 2018). While a handful of records exist for *C. gigantea* (Jayasinghe et al., 2013, Perera and Wickramasinghe, 2014,

Wijeweera et al., 2022), comprehensive studies on its associated fauna remain scarce. Consequently, the current investigation aims to assess the prevalence of insect flower visitors linked to *C. procera* plants in Dumraon district of Buxar, Bihar.

## MATERIAL AND METHODS

**Study area:** The experiment was carried out at College Farm Veer Kunwar Singh College of Agriculture, Dumraon, Buxar under Bihar Agricultural University, Sabour, Bhagalpur during the year 2022-23 and 2023-24. Three sites with high population of *Calotropis procera* (A. Latitude- 84°9'38", Longitude- 25°31'40"; B. Latitude- 84°9'44", Longitude- 25°31'43" and C. Latitude- 84°9'25", Longitude- 25°31'4") were purposively sampled after conducting reconnaissance survey; these were Permanent sites of Haryana farm. The studied area was then mapped using Arc GIS.

**Observations:** Study on diversity and abundance of Insect pollinators on Aak were taken up at three different places of College Farm (Haryana Farm), Veer Kunwar Singh College of Agriculture, Dumraon, Buxar from December to May in each year (Fig. 1). The obtained data were pooled for further study and analysis. The diversity of insect pollinators on *Calotropis procera* was on weekly basis. There the experimental collection sites were regularly visited and samples were collected by using a hand sweeping net from 7 am to 5 pm on every week. Collected samples were preserved in the killing bottles with ethyl acetate and brought

into the laboratory for spreading the insects and photography. Identification of the pollinators was done by matching with previously identified fauna of pollinators

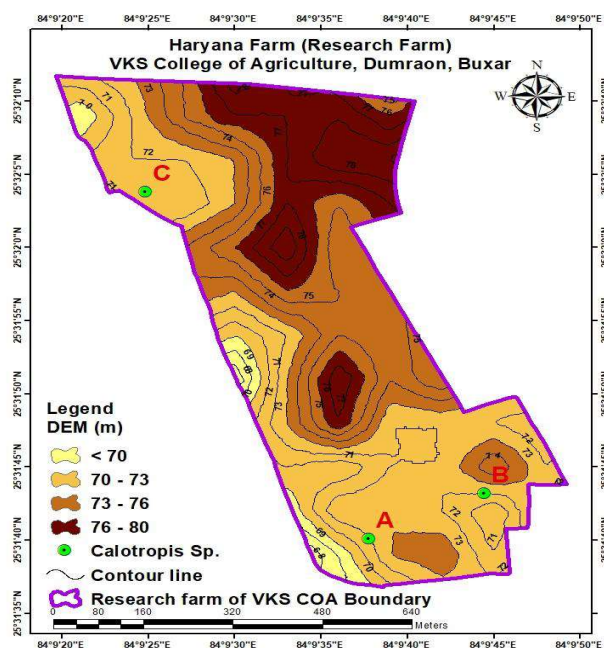


Fig. 1. Location map of studied area

preserved in the Insect Museum, Department of Agricultural Entomology and also with assistance of experts. Relative abundance which determines percentage of specimens of a given species in the total number of organisms collected, were calculated based on the observations taken on the number of pollinators visiting the flowers/m<sup>2</sup>/5 min, using following formula: Relative abundance (%) = population of a particular species visiting flower/total population of all species visiting flowers x 100 (Das and Jha, 2019). The data on relative abundance of pollinators recorded during the studied period were pooled for the analysis. The diversity index to measure the species diversity in a community like Simpson's Index of Diversity and Simpson's Reciprocal Index were worked out based on pooled data based on the methods suggested by Simpson (Simpson 1949).

## RESULTS AND DISCUSSION

During the study period, a total of 21 insect forager species, including two unidentified species, were observed visiting *Calotropis procera* (Table 1). The recorded pollinators belong to five orders viz. Hymenoptera, Diptera, Coleoptera, Lepidoptera, and Orthoptera. Among these orders, the highest numbers of species (12) were observed in the Hymenoptera order, demonstrating greater activity on C.

Table 1. Diversity of insect pollinators on *Calotropis procera* (Aak)

Common name	Scientific name	Order	Family
Ant	<i>Monomorium indicum</i>	Hymenoptera	Formicidae
Megachilid	<i>Megachile lanatus</i>	Hymenoptera	Megachilidae
Rock honeybee	<i>Apis dorsata</i>	Hymenoptera	Apidae
Little honeybee	<i>Apis florea</i>	Hymenoptera	Apidae
Stingless bee	<i>Tetragonula iridipennis</i>	Hymenoptera	Apidae
Carpenter bee	<i>Xylocopa fensterata</i>	Hymenoptera	Xylocopidae
Carpenter bee	<i>X. volvoceae</i>	Hymenoptera	Xylocopidae
Syrphid fly	<i>Episyrphus sp.</i>	Diptera	Syrphidae
House fly	<i>Musca sp.</i>	Diptera	Muscidae
Blister beetle	<i>Mylabris pustulata</i>	Coleoptera	Meloidae
Italian honeybee	<i>Apis mellifera</i>	Hymenoptera	Apidae
Indian honeybee	<i>Apis cerana indica</i>	Hymenoptera	Apidae
Pumpkin beetle	<i>Aulacophora foveicollis</i>	Coleoptera	Chrysomelidae
Pea blue butterfly	-	Lepidoptera	Lycaenidae
Sweat bees	-	Hymenoptera	Halictidae
Ladybird beetle	<i>Coccinella transversalis</i>	Coleoptera	Coccinellidae
Short-horned grasshoppers	-	Orthoptera	Acrididae
Sweat bees	-	Hymenoptera	Halictidae
Plain Tiger Butterfly	<i>Dannus chrysippus</i>	Lepidoptera	Nymphalidae
Unidentified 1	-	Hymenoptera	-
Unidentified 2	-	Diptera	-

**Table 2.** Abundance of pollinators *Calotropis procera*

		Population abundance of pollinators on <i>Calotropis procera</i>												
Common name	Scientific name	Family	December	January	February	March	April	May						
			No./MT <sup>2</sup> /5 min.	Mean (%)	No./MT <sup>2</sup> /5 min	Mean (%)	No./MT <sup>2</sup> /5 min	Mean (%)	No./MT <sup>2</sup> /5 min	Mean (%)	No./MT <sup>2</sup> /5 min	Mean (%)	No./MT <sup>2</sup> /5 min	Mean (%)
Ants	<i>Monomorium indicum</i>	Formicidae	2	7.61	1.75	8.64	3.42	18.41	5.25	10.92	5.58	8.06	4.8	7.87
Megachilids	<i>Megachile lanatus</i>	Megachilidae	0.67	2.55	0.25	1.23	0.58	3.12	0.5	1.04	2.08	3.00	2.93	4.80
Rock honeybee	<i>Apis dorsata</i>	Apidae	1.87	7.12	2.5	12.35	1.08	5.81	4.67	9.71	2.83	4.09	1.53	2.51
Little honeybee	<i>Apis florea</i>	Apidae	1.47	5.60	1.42	7.01	0.08	0.43	4.08	8.48	3.75	5.41	2.13	3.49
Stingless bee	<i>Tetragonula iridipennis</i>	Apidae	1.27	4.83	1.08	5.33	0.67	3.61	1.75	3.64	4	5.78	2.75	4.51
Carpenter bee	<i>Xylocopa fensterata</i>	Xylocopidae	1.13	4.30	1.08	5.33	0.92	4.95	2.67	5.55	1.03	1.49	1.03	1.69
Carpenter bee	<i>X. volvoceae</i>	Xylocopidae	1.53	5.82	1	4.94	1	5.38	3.92	8.15	2.83	4.09	3.13	5.13
Syrphid fly	<i>Episyrphus sp.</i>	Syrphidae	1.73	6.59	0.33	1.63	0.92	4.95	1.83	3.81	3.08	4.45	2.13	3.49
House fly	<i>Musca sp.</i>	Muscidae	1.33	5.06	0.58	2.86	0.58	3.12	1.92	3.99	2.75	3.97	4.6	7.54
Blister beetle	<i>Mylabris pustulata</i>	Meloidae	0.67	2.55	1.83	9.04	0.58	3.12	1.5	3.12	3	4.33	3.87	6.34
Italian honeybee	<i>Apis mellifera</i>	Apidae	0.73	2.78	1.5	7.41	0.67	3.61	1.92	3.99	4.5	6.50	2.75	4.51
Indian honeybee	<i>Apis cerana indica</i>	Apidae	1.73	6.59	0.67	3.31	0.83	4.47	3	6.24	3.58	5.17	4.27	7.00
Pumpkin beetle	<i>Aulacophora foveicollis</i>	Chrysomelidae	0.73	2.78	1.08	5.33	1.08	5.81	2.67	5.55	5.42	7.83	3.8	6.23
Pea blue butterfly	-	Lycaenidae	0.6	2.28	1.58	7.80	0.92	4.95	1.58	3.29	4.25	6.14	3.13	5.13
Sweat bees	-	Halictidae	1.8	6.85	0.75	3.70	0.92	4.95	2.08	4.33	3.67	5.30	4.67	7.66
Ladybird beetle	<i>Coccinella transversalis</i>	Coccinellidae	1.2	4.57	1.25	6.17	0.5	2.69	0.5	1.04	3.08	4.45	2.07	3.39
Short-horned grasshoppers	-	Acrididae	1.4	5.33	0.17	0.84	0.42	2.26	1.67	3.47	3.83	5.53	3.53	5.79
Sweat bees	-	Halictidae	1.2	4.57	0.5	2.47	0.75	4.04	2.33	4.85	3	4.33	2.4	3.93
Plain Tiger Butterfly	<i>Dannus chrysippus</i>	Nymphalidae	0.75	2.85	0.75	3.70	0.83	4.47	0.75	1.56	0.75	1.08	0.75	1.23
Unidentified 1	-	-	0.93	3.54	0.17	0.84	0.58	3.12	2.25	4.68	3.25	4.69	2.07	3.39
Unidentified 2	-	-	1.53	5.82	1.17	5.78	1.25	6.73	1.25	2.60	3	4.33	3.2	5.25
Total			26.27		21.41	18.58	48.09	69.26	61.54					

Note: Mean pooled population abundance of pollinators on *Calotropis procera* during 2022-23 and 2023-24

**Table 3.** Diversity index of insect pollinators on *Calotropis* spp

Months	Location A			Location B			Location C		
	Latitude: 25°31'40", Longitude: 84°09'38"			Latitude: 25°31'43", Longitude: 84°09'44"			Latitude: 25°31'40", Longitude: 84°09'25"		
	(D)	(1-D)	(1/D)	(D)	(1-D)	(1/D)	(D)	(1-D)	(1/D)
December	0.051	0.949	19.677	0.049	0.951	20.263	0.052	0.948	19.408
January	0.051	0.949	19.679	0.052	0.948	19.239	0.049	0.951	20.533
February	0.051	0.949	19.468	0.048	0.952	21.000	0.054	0.946	18.600
March	0.052	0.948	19.404	0.049	0.951	20.545	0.054	0.946	18.533
April	0.058	0.942	17.251	0.053	0.947	19.024	0.045	0.955	22.165
May	0.048	0.952	20.744	0.043	0.957	23.290	0.047	0.953	21.223

Simpson's Index (D), Simpson's Diversity Index (1-D), Simpson's Reciprocal Index (1/D)

*procera* compared to other insect groups. In contrast, only one species from the Orthoptera order was identified foraging on the plants. Conversely, three species from both Diptera and Coleoptera orders, followed by two species from Lepidoptera, were observed visiting the plant (Fig. 1). When the family wise relative abundance of insect pollinators was taken into consideration it is observed that maximum no. of species visiting *C. Procera* belongs to the family Apidae followed by two species of both Xylocopidae and Halictidae. (Wijeweera et al., 2022) documented *Xylocopa* spp. (Carpenter bee), *A. cerana* Fabricius (Honeybee), and *D. chrysippus* (Plain Tiger) were commonly recorded as pollinators of *C. Gigantea*. Charan et al. (2020) reported that *C. procera* attracted 27 insect pollinators from three orders and 14 tribes in the Thar Desert of Rajasthan. Minor variations in the findings could be attributed to differences in geographical locations and climatic conditions. Baghele and Masram (2023) observed 18 insect species on the plant during the summer season. Among these, five belonged to Order Hemiptera, four to Diptera, three to Coleoptera, two to Lepidoptera, two to Hymenoptera, and two to Araneae, with the potential for acting as pollinators in close proximity to the present study. Baburao (2021) documented sixty-two insect species associated with milkweed, representing nine orders, 40 families, and 54 genera. Of these, 17.74% of the species were identified as floral visitors.

Highest mean population of pollinators was observed in April (69.26/m<sup>2</sup>/5 minute) followed by May, December and January. The lowest was observed in February (18.58 / m<sup>2</sup>/5 minute). Ants were most promising visitors on the plant during all months of study except in January. In this month *Apis dorsata* was the most frequent visitors (2.5 /m<sup>2</sup> /5 min) visited the flowers for predation of soft bodied insects and unknowingly pollinates the flowers. Minimum number and percent mean of population of pollinators December to May was Pea blue butterfly. Charan et al. (2020) documented

*Trigona iridipennis* to be the most abundant (18.85 / m<sup>2</sup>/ 5 minutes) and *Chrysosoma* spp (1.44 / m<sup>2</sup>/ 5 minutes) as the least abundant visitor in the summer while *Apis florea* as the most abundant (14.70 /m<sup>2</sup>/ 5 minutes) pollinator in the winter season. On the basis of insect foragers recorded, mean number of pollinators per m<sup>2</sup> per 5 minutes, Hymenoptera has been observed as the most abundant order while Apidae as the most abundant family.

Simpson's index spans from 0 to 1, where a value of zero signifies lower species diversity, while one indicates a richness of diversity. According to the Simpson's index *Calotropis* areas exhibited a high richness of pollinator diversity (ranging from 0.942 to 0.955) throughout the study months (Table 3). The maximum species richness values were noted at location C in March (0.955) conserving pollinators with weeds can offer an alternative to addressing threats to biodiversity, consistent with findings by Steffan-Dewenter and Westphal (2008). Rollin et al. (2016) also revealed that despite being formidable competitors, weeds contribute to floral diversity in agricultural landscapes and provide food for insects in exchange for pollination. Chatterjee et al. (2020) predicted thresholds of 27% and 18% for natural vegetation cover to safeguard pollinator services in brinjal and mustard, respectively. Rollings and Goulson (2019) further support this perspective.

## CONCLUSION

The study identified a diverse array of insect pollinators visiting *Calotropis procera*, with a total of 21 species across five orders. The Hymenoptera order exhibited the highest species richness, underscoring its significant role in the pollination of *C. procera*. Apidae was being the most prevalent. Ants emerged as the most consistent visitors, except in January when *Apis dorsata* was the most frequent. This research investigates the importance of conserving pollinator habitats, including weedy species like *C. procera*,



which play a crucial role in maintaining biodiversity and ecosystem services. The study's findings are consistent with broader ecological perspectives that highlight the contribution of weeds to floral diversity and their potential in supporting pollinator communities.

#### AUTHORS CONTRIBUTION

Gautam Kunal planned the design of the study, carried out the experiments and drafted the manuscript. Jitendra Kumar conceptualized the work and collected the data. Love Kumar contributed in mapping and statistical analysis of the data. Ramesh Nath Gupta reviewed and corrected the manuscript. All authors read and approved the final manuscript.

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# Impact of Extrafloral Nectaries of *Impatiens balsamina* L. on Incidence of *Myrmicaria brunae* and *Camponotus rufoglaucus*

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**Abstract:** Morphotypes of extrafloral nectaries were elevated at petioles, basal portion of leaf margins; paired, stalked button shaped in petioles, basal portion of leaf margins, smooth surfaced and bigger on petioles, smaller on leaf margins on *Impatiens balsamina* L. The number of EFNs were maximum and minimum at 36<sup>th</sup> and 42<sup>nd</sup> standard week in all the three varieties respectively. Population of *Myrmicaria brunae* was higher at 35<sup>th</sup>, 34<sup>th</sup>, 36<sup>th</sup> standard week. Population of *Camponotus rufoglaucus* J. were maximum at 36<sup>th</sup> standard week in variety 1 -purple and variety 2 – white and 35<sup>th</sup> standard week in variety 3 -purple with white. Population of *M. brunae* and *C. rufoglaucus* were lowest at 42<sup>nd</sup> standard week in all the three varieties. In *I. balsamina*, significantly positive correlation was observed between *M. brunae*, *C. rufoglaucus* and EFN numbers. Significantly positive correlation was also observed between *M. brunae* and *C. rufoglaucus* in variety 1,3 but were non-significant in variety.

**Keywords:** Extrafloral nectaries, *Impatiens balsamina*, *Myrmicaria*, *Camponotus*

Balsam (*Impatiens balsamina* L.) also known as jewel weed, snap weed or touch-me-not belongs to the family Balsaminaceae. It is an annual plant growing up to 20-75cm tall with a thick, but succulent stem, native to India and Myanmar (USDA-ARS 2018). Balsam is mainly cultivated for its medicinal properties and also grown as ornamental. From roots to seeds, all parts of balsam have medicinal properties (Meenu et al., 2015). Balsaminaceae family which includes genus *Impatiens* contains extrafloral nectaries glands at petioles as stalk shaped (Nalini et al., 2019). Extrafloral nectar (EFN) is a liquid material rich in carbohydrates with a minimum amount of amino acids, lipids, phenols, alkaloids and volatile organic compounds (Nalini and Sneka 2021). The presence of EFN glands in *I. balsamina* were identified by many authors (Nalini et al., 2019) but the morphology of EFN glands differ from one another. Studies are scanty on the evaluation of the impact of EFNs on ant population in *I. balsamina*. So present study was initiated to throw light on it.

## MATERIAL AND METHODS

Experiments were conducted in balsam plants during *Kharif* 2021 and *Rabi* 2021-2022 in the pots at the pot culture yard of Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu. No pesticides were sprayed on the crops throughout the study period. The balsam plant was selected for the presence of more extrafloral nectaries (EFNs). The balsam seeds [variety 1 (purple), variety 2 (white), variety 3 (purple with white)] were

procured from Tamilachi ornamental garden located at Kanyakumari district.

**Morphology and distribution of extrafloral nectaries:** Plant parts like stem, petiole, leaves, calyx, buds, bract, bracteoles, on parts of inflorescence, flower stalks and fruits were checked thoroughly for the presence of extrafloral nectaries (EFNs) structures in balsam of age 30 DAS under stereo zoom microscope (Stemi DV4, Zeiss) at 10x magnification (Nalini et al., 2019). EFNs were identified by their conspicuous raised glands or recessed basins and are sometimes coloured differently than the surrounding plant material. Morphotype, location, distribution, shape and surface of EFNs on different plant parts were recorded and photographed (Mortazavi et al., 2017).

**Effect of EFNs on ants:** In the three varieties of balsam, the number of EFNs (visible as a glossy surface) were counted at petiole and basal portion of leaf margin. The number of ants were recorded at 7-11 am (lasting five minutes in each plant) from one month after sowing at weekly intervals (12 counts). Each treatment was replicated ten times. The ants collected with aspirator were stored in 75% ethyl alcohol (Nalini and Sneka, 2021). Identification of preserved ants to species level was done in the Department of Entomology, Faculty of Agriculture, Annamalai University following the taxonomic keys of Bolton (1994); Tiwari (1999) and Hashimoto (2003) using Stemi DV4 Stereo (Zeiss) microscope.

## RESULTS AND DISCUSSION

**Morphology and distribution of extrafloral nectaries in *I.***

**balsamina:** Morphotypes of extrafloral nectaries (EFNs) on *I. balsamina* were elevated at petioles and basal portion of leaf margins. EFNs were paired, stalked button shaped on petioles and basal portion of leaf margins. EFNs in *I. balsamina* are smooth surfaced. EFNs sizes were bigger on petioles and smaller on leaf margins. On *I. balsamina* morphotypes of extrafloral nectaries (EFNs) were elevated at petioles and basal portion of leaf margins. EFNs were paired, stalked button shaped in petioles and basal portion of leaf margins. EFNs in *I. balsamina* was smooth surfaced. EFNs sizes were bigger on petioles and smaller on leaf margins. EFNs colours were reddish brown, dark green and pale green in variety 1, 2, 3 respectively. Based on the colour of the flowers, *I. balsamina* was treated as variety 1 (purple), variety 2 (double coloured), variety 3 (white).

Extrafloral nectaries (EFN), nectar-producing glands can be found on leaf laminae, petioles, rachids, bracts, stipules, pedicels, fruit, and other parts of the plant, and their size,

form, and secretions differ depending on the taxon (Mizell 2001). The *I. balsamina* has extrafloral nectaries on its leaves and petioles. Extrafloral nectaries in plants of Hong Kong were examined by So (2004). Button-shaped, cup-shaped, stalk-shaped, pit-shaped, and pore shaped are the five major varieties reported on them. The Euphorbiaceae family contains the most extrafloral nectaries, which are always visible structures that attract ants. The extrafloral nectaries of *I. balsamina* are located as paired and stalk shaped on petioles. These observations confirmed the findings of the present study. Nalini et al. (2019) conducted a survey at Annamalai Nagar and Sivapuri. Out of 162 plant species EFNs were identified in 62 plants and listed the morphology and distribution of EFNs for 62 plants for the first time in India and explained that extrafloral nectaries of *I. balsamina* are present on the abaxial surface of the leaf on the lamina and stalked button form at petioles.

**Effect of extrafloral nectaries on ants:** The number of

Crop name	Morphotype	Location	Distribution	Size	Shape	Surface
Balsam	Elevated nectaries	Petioles and basal portion of leaf margins	Paired	Big (petioles) and small (leaf margin)	Stalked and button shaped	Smooth

**Table 1.** Effect of EFNs on incidence of *Myrmicaria brunae* and *Camponotus rufoglaucus* in *Impatiens balsamina*

Standard week	Mean number of EFNs/plant*			Mean number of <i>M. brunae</i> /plant*			Mean number of <i>C. rufoglaucus</i> /plant*		
	Variety 1	Variety 2	Variety 3	Variety 1	Variety 2	Variety 3	Variety 1	Variety 2	Variety 3
31	118.96 (10.82)	81.12 (8.94)	75.84 (8.64)	7.43 (2.71)	6.29 (2.49)	2.94 (1.70)	0.63 (0.79)	0.43 (0.65)	0.29 (0.53)
32	123.20 (11.01)	84.44 (9.12)	78.72 (8.80)	8.83 (2.95)	7.43 (2.71)	4.29 (2.06)	1.14 (1.06)	0.37 (0.60)	0.63 (0.79)
33	128.38 (11.24)	85.84 (9.19)	80.84 (8.92)	18.57 (4.28)	14.43 (3.77)	4.49 (2.10)	1.26 (1.11)	0.43 (0.65)	0.80 (0.89)
34	132.16 (11.41)	89.36 (9.38)	84.00 (9.09)	18.63 (4.28)	15.31 (3.88)	4.66 (2.14)	1.86 (1.35)	0.71 (0.84)	0.57 (0.75)
35	137.28 (11.63)	94.40 (9.64)	89.44 (9.38)	19.29 (4.36)	13.06 (3.59)	7.06 (2.64)	1.34 (1.15)	1.49 (1.21)	0.83 (0.90)
36	144.52 (11.93)	98.48 (9.85)	94.32 (9.64)	16.43 (4.02)	12.11 (3.45)	7.43 (2.71)	2.14 (1.45)	1.77 (1.32)	0.51 (0.71)
37	136.64 (11.60)	88.00 (9.31)	84.32 (9.11)	17.54 (4.16)	11.80 (3.41)	6.11 (2.45)	1.37 (1.16)	0.92 (0.95)	0.69 (0.82)
38	131.92 (11.40)	75.84 (8.64)	68.96 (8.24)	15.97 (3.97)	11.34 (3.34)	5.69 (2.37)	1.21 (1.09)	0.74 (0.85)	0.55 (0.74)
39	126.04 (11.14)	71.60 (8.40)	64.84 (7.99)	14.83 (3.82)	10.06 (3.15)	3.88 (1.95)	1.10 (1.04)	0.52 (0.72)	0.39 (0.62)
40	119.12 (10.83)	69.76 (8.29)	60.08 (7.69)	11.54 (3.37)	6.66 (2.56)	1.83 (1.34)	0.99 (0.99)	0.34 (0.58)	0.29 (0.53)
41	97.2 (9.78)	59.4 (7.65)	51.28 (7.11)	10.06 (3.15)	7.11 (2.65)	0.54 (0.73)	0.78 (0.88)	0.32 (0.56)	0.25 (0.50)
42	84.4 (9.12)	55.2 (7.37)	47.12 (6.81)	7.31 (2.68)	5.49 (2.33)	0.48 (0.69)	0.63 (0.79)	0.26 (0.51)	0.23 (0.48)
Mean	10.99	8.81	8.45	3.64	3.11	1.91	1.07	0.79	0.69
CD (p=0.05)		(0.05)			(0.03)			(0.01)	

\*Mean of 10 replications. Values in parentheses are transformed values  $\sqrt{x+0.5}$   
Variety 1 -purple, Variety 2 - white, Variety 3 -purple with white

EFNs were significantly highest (144.52, 98.48, 94.32) at 36<sup>th</sup> standard week followed by 35<sup>th</sup> standard week in variety 1, 2, 3 respectively. The number of EFNs were significantly lowest at 42<sup>nd</sup> standard week followed by 41<sup>st</sup> week. Number of *M. brunae* were significantly highest at 35<sup>th</sup> standard week followed by 34<sup>th</sup> standard week. Number of *M. brunae* were significantly lowest at 42<sup>nd</sup> standard week. Numbers of *C. rufoglaucus* were significantly highest at 36<sup>th</sup> standard week in variety 1 and 2 and 35<sup>th</sup> standard week in variety 3 respectively followed by 34<sup>th</sup>, 35<sup>th</sup>, 33<sup>rd</sup> standard weeks. Number of *C. rufoglaucus* were significantly lowest at 42<sup>nd</sup> standard week (Table 1).

The number of EFNs were highest and lowest at 36<sup>th</sup> and 42<sup>nd</sup> standard week in all the three varieties. The number of *M. brunae* were highest at 35<sup>th</sup> standard week in variety 1, 34<sup>th</sup> standard week in variety 2 and 36<sup>th</sup> standard week in variety 3 respectively. The number of *C. rufoglaucus* were highest at 36<sup>th</sup> standard week in variety 1, 2 and 35<sup>th</sup> standard week in variety 3. The number of *M. brunae* and *C. rufoglaucus* were lowest at 42<sup>nd</sup> standard week in all the three varieties (Table 1).

Among the three varieties, variety 1 showed highest EFN numbers than variety 2 and 3. The number of EFNs in *I. balsamina* showed increasing pattern from 31<sup>st</sup> to 36<sup>th</sup> standard week but from 37<sup>th</sup> standard week it showed decreasing pattern up to 42<sup>nd</sup> standard week. As the EFNs of *I. balsamina* were present on petiole and leaf margins their number were more during peak of vegetative stage (34<sup>th</sup> to 36<sup>th</sup> standard weeks) in all the three varieties. Also, both ant species (*M. brunae* and *C. rufoglaucus*) were found highest in number on plants during the same period (34<sup>th</sup> to 36<sup>th</sup> standard weeks) which shows *I. balsamina* attracted them to safeguard their vegetative parts which are crucial for further flower production. Significantly positive correlation was observed between *M. brunae* (0.764, 0.713, 0.903), *C. rufoglaucus* (0.779, 0.747, 0.769) and EFN numbers in all the three varieties. *M. brunae* and *C. rufoglaucus* were significantly positively correlated in variety 1 and 3 (0.745, 0.787) but were non-significant in variety 2 (0.560) (Table 2).

Nogueira et al. (2012) investigated and found that the proportion of plants tended by ants in each plot was higher in drier not wetter months and at the plant level, ant attendance

increased strongly with the number of recently formed shoot nodes, and ants almost never attended plants with limited or no young tissue. Some plant species secrete large amounts of EF nectar from young or middle-aged leaves and less nectar from old leaves (Yamawo et al., 2012), attracting numerous ants to important parts for their growth. This is in accordance to the present study results. Extrafloral nectar is a nutritive resource rich in sugar and amino acids which has a direct impact on ant activity on plants and may improve ant colony fitness (Byk and Del-Claro, 2011). Mohankumar and Nalini (2016) identified ant species such as *Camponotus rufoglaucus* and *Myrmecaria brunae*. For *Mallotus japonicus* plants in the field, most mutualistic ant species such as *Pheidolenoda* and *Crematogaster teranishii* are more frequently observed on EF nectar-rich plants than on other plants, and non-aggressive ant species are observed to visit the plants having less EF-nectar (Yamawo et al., 2014, 2017). These literatures are in line with the present findings.

Several studies narrated the importance of EFNs in sustaining the multitrophic interactions which stated that: EFNs are produced by at least 4,000 plant species throughout the world (Weber and Keeler, 2013). Their nectar functions to attract ants which then repel or kill insect herbivores (Del-Claro et al., 2016, Heil, 2015) and increasing plant growth and reproductive success (Marazzi et al., 2013). Extrafloral nectar implicates the structure and composition of ant communities and for multitrophic ant-plant-herbivore interactions (Dattilo et al., 2015, Lange et al., 2017; Lange et al., 2013). Increased EF nectar production increases ant activity and aggressiveness and is considered to favor mutualistic ants rather than parasitic ones (Heil, 2013). This partner choice by plants has been considered to be an effective mechanism for stabilizing ant-plant mutualisms (Grasso et al., 2015). Daily variation ant foraging occurred only on plants bearing EFNs. The recruitment of ants to plants with EFNs was five times higher compared to plants without EFNs, with which several species did not interact. Several studies show reduced numbers of ants interacting with plants naturally lacking EFNs compared to plants bearing EFNs in the Brazilian savanna (Maravalhas and Vasconcelos 2014).

**Table 2.** Simple correlation matrix between EFNs number and different ant species in *Impatiens balsamina*

Parameters	EFNs no.			<i>M. brunae</i>			<i>C. rufoglaucus</i>		
	Variety 1	Variety 2	Variety 3	Variety 1	Variety 2	Variety 3	Variety 1	Variety 2	Variety 3
EFNs no.	1	1	1	-	-	-	-	-	-
<i>M. brunae</i>	0.764**	0.713**	0.903**	1	1	1	-	-	-
<i>C. rufoglaucus</i>	0.779**	0.747**	0.769**	0.745**	0.560	0.787**	1	1	1

\*\*Significant at 0.01 probability level, variety 1 (purple), variety 2 (white), variety 3 (purple with white)]

## CONCLUSION

Morphotypes of extrafloral nectaries (EFNs) on *I. balsamina* were elevated at petioles and basal portion of leaf margins. EFNs were paired, stalked button shaped on petioles and basal portion of leaf margins and smooth surfaced. EFNs sizes were bigger on petioles and smaller on leaf margins. Both ant species (*M. brunae* and *C. rufoglaucus*) were highest number on plants during the same period (34<sup>th</sup> to 36<sup>th</sup> standard weeks) which shows *I. balsamina* attracted them to safeguard their vegetative parts which are crucial for further flower production.

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# Unveiling the Insect Spectrum of Carrot (*Daucus carota* L.) in Eastern Dry Zone of Karnataka

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**Abstract:** The study investigates the insect fauna associated with carrot crops in the Eastern Dry Zone of Karnataka, India. Roving surveys conducted across Kolar, Chikkaballapur and Bengaluru Rural districts resulted in documenting a total of 39 insect species across four orders and 12 families, where Hemiptera order being the most diverse and order Diptera being most abundant. Major pests identified include leafminer, *Liriomyza trifolii*, carrot fly, *Melanagromyza* sp., mirid bug, *Taylorilygus apicalis*, Lygaeid bug, *Nysius* sp., leafhoppers, *Balclutha* spp. and *Hishimonus phycitis* which cause substantial damage on carrots. All these insects are being newly recorded on carrot in India. *Liriomyza trifolii* and *Melanagromyza* sp. were the most economically significant pests causing 42.33% damage to foliage and 20.77% to taproots respectively. Species diversity indices revealed Kolar as the most diverse district, followed by Bengaluru Rural and Chikkaballapur.

**Keywords:** Carrot, Insect fauna, *Liriomyza trifolii*, *Melanagromyza* sp.

Carrot (*Daucus carota* L.) is one of the ten leading vegetables grown globally, about 1.2 million hectares of land are currently used for carrot cultivation in the world with an estimated economic value of nearly \$14 billion (Bolton et al., 2020). Originating from Afghanistan and Persia, carrots belong to the Apiaceae family. China holds the top position globally as the leading producer of carrots and turnips, producing 21.3 million tonnes. India, occupies the 14<sup>th</sup> position, covering a total area of 0.122 million hectares, with production of 2.3 million metric tonnes. Predominantly carrot is a temperate climate plant but, it is also cultivated in tropical and subtropical regions, especially at high elevations with assured irrigation.

Carrots being a high value vegetable crop have attracted many growers in different parts of Karnataka and neighbouring states. Like other vegetable crops, carrots have also been infested by many insect pests. Insect pests damaging carrot include carrot rust fly, *Psila rosae* (F.), aster leafhopper, *Macrostelus quadrilineatus* (Fobes), flea beetle, *Systema blanda* (Melsheimer), carrot weevil, *Listronotus oregonensis* (LeConte), aphid, *Myzus persicae* (Sulzer) and cutworm, *Agrotis* sp. from United States of America. Collier (2017) reported several species of aphid viz., *Cavariella aegopodi* (Scopoli), *C. pastinacae* (Linnaeus), *C. theobaldi* (Gillete and Bragg), *Myzus persicae* (Sulzer) and turnip moth, *Agrotis segetum* (Denis and Schiffermuller) as major pests of carrot from England. Bhat and Ahangar (2018) recorded four insects on carrot which includes carrot aphid, *Cavariella aegopodii* (Scopoli), *Semiaphis heraclei*

(Takahashi); semilooper, *Thysanoplusia orichalcea* (Fabricius) and thrips, *Aeolothrips meridionalis* Bagnall from Jammu and Kashmir, India. The current study was aimed to investigate the insect fauna on carrot crop, their distribution, abundance, diversity and its potential on carrots in the Eastern dry zone of Karnataka.

## MATERIAL AND METHODS

Roving surveys were conducted in major carrot growing districts of Karnataka viz., Kolar, Chikkaballapur and Bengaluru Rural during December 2021-22 to December 2022-23 to document the insect fauna of carrots. Fields were visited at fortnightly intervals, with each field being sampled twice for observations, and unsprayed fields were selected for the study. In total, 30 fields were surveyed during the study period, with samples taken from carrot crops at various developmental stages. Since the duration of carrot crops is 85-90 days, the entire crop period was divided into five developmental stages: stage I (1-25 days), stage II (26-40 days), stage III (41-60 days), stage IV (61-75 days), and stage V (76-90 days) (Wang et al., 2016). Samplings for insect fauna were made in the field by sweep netting on carrot foliage by adapting the zig-zag sampling method. Insects along with the foliage were immediately collected using polythene bags. Ten samples were collected in 0.4 ha area and each sample was a composite of five sweeps. Insect abundance recorded by sweep netting was expressed as an average number of adults per sample. Simultaneously, the abundance of aphids and lepidopteran caterpillars on the leaf

was recorded by counting the total number of aphids and caterpillars on ten randomly selected plants in each quadrant. A total of ten quadrats were observed and abundance was expressed as the mean number of insects per plant.

Pestiferous and beneficial insects associated with carrots in different surveyed locations were collected and brought to the laboratory, and the immature stages were reared till the adult stage. The adult insects were preserved for taxonomic identification. Soft-bodied insects were preserved in 70% ethanol. The insect faunal abundance and species diversity were worked out by using different indices such as Margalef's index of richness (MI), Shannon-Wiener Diversity index (H), Simpson's index (D), Pielou's evenness index (J) and Berger-Parker index of dominance (d). Engelmann's scale of dominance was used to evaluate the dominance structure of the ecosystem. Based on relative abundance, the dominance structure was categorized into five scales as follows: eudominant (>31.7%), dominant (10.1–31.6%), subdominant (3.2-10%), recedent (1.1-3.1%) and subrecedent (<1%).

## RESULTS AND DISCUSSION

The 39 insect species belonging to four orders and 12 families were found associated with carrot crops in three major carrot-growing districts of Karnataka (Table 1). Order Hemiptera was the most diverse with 33 species documented, followed by Diptera with four species, and one species each from Coleoptera and Lepidoptera. Family Cicadellidae (Hemiptera) was the most speciose representing 14 species, followed by Delphacidae (eight species), Miridae (five species), Agromyzidae (two species), Pentatomidae and Tephritidae with two species each. Noctuidae, Coreidae, Lygaeidae, Geocoridae, Chrysomelidae and Aphididae with one species each. This is a maiden attempt in India to document the insects associated with carrots. Consequently, a thorough examination of the nature of damage inflicted by major insect pests on carrots has been meticulously documented and discussed herein.

During the initial stages of crop growth, the presence of leafhoppers and planthoppers leads to the complete yellowing of foliage (Fig. 1a and 1b), consequently impeding plant growth. Among the Cicadellidae family, *Balclutha* spp. emerged as the dominant leafhoppers, followed by *Hishimous phycitis* (Distant) and *Empoasca* sp. All four species were consistently observed across all surveyed locations. In a study conducted in Michigan, USA (Stillson and Szendrei 2020), researchers investigated leafhoppers in carrot crops and identified a total of 23 taxa. Among these, *Balclutha* sp. and *Empoasca fabae* (Harris) were noted as two of the eight

most prevalent leafhopper taxa, which is in accordance with this study. Among the Delphacidae family *Toya* sp1. was the sole predominantly observed planthopper. Wilson and O'Brien (1987) in the Australo-Pacific regions, also recognized *Toya dryope* (Kirkaldy) and *Sogatella kolophon* (Kirkaldy) as pestiferous planthoppers affecting carrots.

Of the five recorded species of Miridae, *Taylorilygus apicalis* (Fieber) was the most prevalent phytophagous mirid bug followed by *Campylomma livida* Reuter and *Halticus minutus* Reuter. The feeding damage by mirid bugs resulted in production of pale whitish circular punctures on the lower surface of carrot leaves, which further results in yellowing of the leaves. *Dortus primaries* Distant and *Nesidocoris tenuis* Reuter are non-pestiferous insects that are general predators of soft-bodied insects and zoophytophagous predators respectively (Sanchez and Lacasa 2008). In India, both *T. apicalis* and *C. livida* are recorded as an important emerging mirids on cotton and sunflower (Rajesh et al., 2020 and Sahana et al., 2023).

Carrots encountered infestation from two agromyzid pests, among which the leafminer, *Liriomyza trifolii* (Burgess), holds substantial importance as a primary defoliator. This pest is known for causing unique serpentine feeding galleries on carrot foliage (Fig. 1c), and a severe infestation can lead to the yellowing and eventual drying of leaves. The attack of leaf miners typically initiates on cotyledon leaves and persists throughout the crop's growth until harvest. Although the initial damage by the leafminer is observed mainly on the lower leaves, if left unchecked, it can extend to infest the entire foliage, leading to a direct impact on the carrot yield. *L. trifolii* was also recorded on carrots in Florida and Brazil (Fernandes et al 2016), but not documented on carrot crops in India.

The carrot fly, *Melanagromyza* sp., is a newly identified pest seen affecting carrots in India (Raghunandan and Manjunatha 2023). The maggot of the carrot fly directly feeds the taproot, creating tunnels in a spherical pattern (Fig. 1d). This ultimately leads to the cracking of carrots, posing a direct impact on both the development and overall quality of the carrot crop. Leaf miners and carrot flies stand out as the main perpetrators responsible for carrot damage, with leaf miners affecting the foliage and carrot flies primarily targeting the taproots. These pests have become the most economically significant threats to carrot cultivation in the Eastern Dry Zone of Karnataka. The defoliation of carrots is also attributed to two other pests, namely the flea beetle, *Monolepta signata* Chevrolat and the semilooper, *Thysanoplusia orichalcea* (F.). *S. blanda* is a globally recognized flea beetle affecting carrots (Nunez and Haviland 2014) but has not been reported in India.

**Table 1.** Insect faunal distribution on carrot in Karnataka

Order	Family	Scientific name	Abundance			Mean	Relative abundance (%)	
			Kolar	Chikkaballapur	Bengaluru rural			
Hemiptera	Cicadellidae	<i>Hishimonus phycitis</i> (Distant)	0.18	0.20	0.20	0.19	2.51	
		<i>Cicadulina bipunctata</i> (Malichar)	0.02	0	0.02	0.01	0.17	
		<i>Balclutha</i> sp1.	0.75	0.77	0.87	0.79	10.35	
		<i>Balclutha</i> sp2.	0.15	0.62	0.41	0.39	5.11	
		<i>Nirvana pallida</i> Malichar	0.09	0	0.05	0.04	0.60	
		<i>Maistes pruthi</i> (Metcalf)	0.01	0.07	0	0.02	0.34	
		<i>Kolla ceylonica</i> (Melichar)	0.03	0	0.01	0.01	0.17	
		<i>Empoasca</i> sp.	0.05	0.02	0.15	0.07	0.95	
		<i>Exitianus indicus</i> (Distant)	0.03	0	0	0.01	0.12	
		<i>Austroagallia nitobei</i> (Matsumura)	0.03	0	0.02	0.01	0.21	
		<i>Thomsonia arcuata</i> (Signorat)	0.03	0	0	0.01	0.12	
		<i>Nephotettix virescens</i> (Distant)	0.04	0	0.02	0.02	0.25	
		<i>Balcluta rubrostriata</i> (Malichar)	0	0.15	0.06	0.07	0.90	
		<i>Seriana</i> sp.	0.02	0	0	0.006	0.08	
		Delphacidae	<i>Toya</i> sp.1.	0.05	0.05	0.07	0.05	0.73
	<i>Cemus</i> sp.		0.02	0	0	0.006	0.08	
	<i>Perkinsiella sinensis</i> Kirkaldy		0.02	0	0	0.006	0.08	
	<i>Coronacella sinhalana</i> (Kirkaldy)		0.05	0	0	0.01	0.21	
	<i>Toya</i> sp.2.		0.02	0	0.05	0.02	0.30	
		<i>Sordia</i> sp.	0.02	0.2	0	0.07	0.95	
Hemiptera	Delphacidae	<i>Sogatella kolophon</i> (Kirkaldy)	0.05	0	0.05	0.03	0.43	
		<i>Tagosodes pusanus</i> (Distant)	0.03	0	0	0.01	0.12	
	Miridae	<i>Taylorilygus apicalis</i> (Fieber)	0.37	0.1	0.5	0.32	4.20	
		<i>Halticus minutus</i> Reuter	0	0	0.07	0.02	0.30	
		<i>Dortus primaries</i> Distant*	0.12	0	0.11	0.07	0.99	
		<i>Campylomma livida</i> Reuter	0.04	0.12	0.02	0.06	0.77	
		<i>Nesidocoris tenius</i> Reuter*	0.05	0.1	0.03	0.06	0.77	
	Coreidae	<i>Cletus</i> sp.	0.04	0	0	0.01	0.17	
	Lygaeidae	<i>Nysius</i> sp.	0.37	0.97	0.03	0.45	5.93	
	Geocoridae	<i>Geocoris</i> sp.	0.04	0.02	0.03	0.03	0.38	
	Pentatomidae	<i>Nezara viridula</i> (L.)	0.09	0.07	0.16	0.10	1.38	
		<i>Menida</i> sp.	0.03	0	0	0.01	0.12	
	Aphididae	<i>Aphis</i> sp.	0.10	0.06	0.02	0.06	0.77	
	Diptera	Tephritidae	<i>Sphenella</i> sp.	0.08	0.12	0	0.06	0.86
			<i>Scadella</i> sp.	0.04	0	0.02	0.02	0.25
Agromyzidae		<i>Melanagromyza</i> sp.	0.14	0.27	0.36	0.25	3.33	
		<i>Liriomyza trifolii</i> (Burgess)	1.55	8.00	2.67	4.07	52.9	
Coleoptera	Chrysomelidae	<i>Monolepta signata</i> Chevrolat	0.12	0.05	0.11	0.09	1.21	
Lepidoptera	Noctuidae	<i>Thysanoplusia orichalcea</i> (F.)	0.02	0.08	0.05	0.05	0.64	

\*Beneficial insects



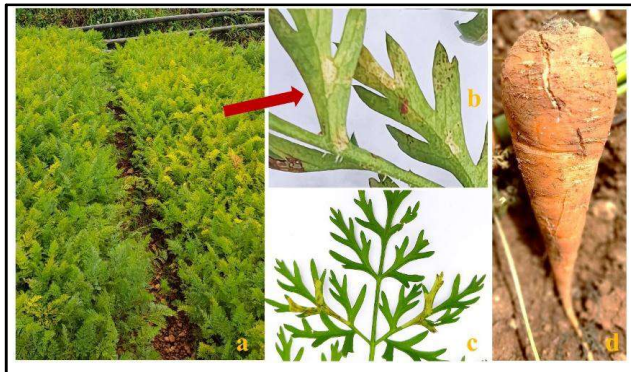
**Table 2.** Diversity of insect fauna of carrot in Eastern Dry Zone of Karnataka

Locality	Number of species (S)	Total number of individuals (N)	Margalef's index of richness (MI)	Shannon's Wiener Diversity index (H)	Simpson's index (1-D)	Pielou's evenness index (J)	Berger parker index of dominance ( $P_{max}$ )
Kolar	37	856	5.33	2.69	0.86	0.39	0.69
Chikkaballapur	20	484	3.07	1.45	0.54	0.21	0.33
Bengaluru rural	27	473	4.22	2.12	0.75	0.30	0.54

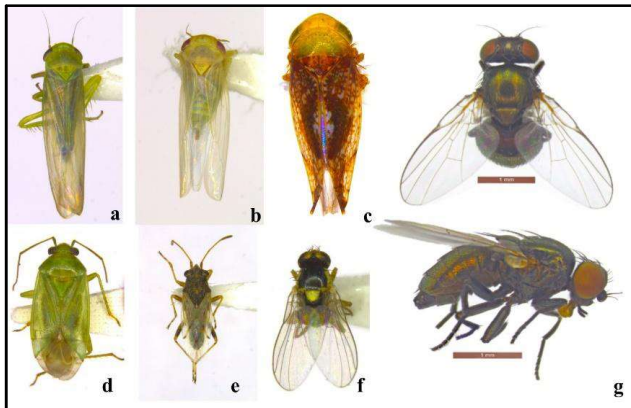
**Table 3.** Succession of major insect pests of carrot with crop developmental stages

Insect pests	Developmental stage of crop (Days)				
	I (0-25)	II (26-40)	III (41-60)	IV (61-75)	V (76-90)
	Germination	Taproot initiation	Taproot development	Pre-maturity	Maturity
<i>L. trifolii</i>	—————				
<i>Balclutha</i> spp.	—————				
<i>Nysius</i> sp.	—————				
<i>T. apicalis</i>	—————				
<i>Melanagromyza</i> sp.	—————				
<i>H. phycitis</i>	—————				

\*The duration of occurrence of each insect pest on the crop is shown by horizontal lines.



**Fig. 1.** Symptoms of damage caused; a and b: sucking insects on foliage; c: leaf miner; d: carrot fly



**Fig. 2.** a: *Balclutha* sp1; b: *Balclutha* sp2; c: *Hishimonus phycitis*; d: *Taylorilygus apicalis*; e: *Nysius* sp.; f: *Liriomyza trifolii*; g: *Melanagromyza* sp.

Considering population densities, relative abundance and nature of damage the following insect species namely *L. trifolii*, *Balclutha* spp., *Nysius* sp., *T. apicalis*, *Melanagromyza* sp. and *H. phycitis* (Table 1, Fig. 2) were considered as major pests on carrots. The progression of major insect pests on carrots was carefully observed and recorded throughout all stages of crop development (Table 3). The richness index was maximum from Kolar, followed by Bengaluru Rural and was minimum for Chikkaballapur (Table 2). The Shannon's Wiener Diversity index and Simpson's index followed a similar trend. Engelmann's classification was used to know the species dominance structure (Dalal and Gupta 2016). Out of 39 insect species recorded in the entire eastern dry zone of Karnataka only one species was found to be eudominant (*L. trifolii*), one species was dominant (*Balclutha* sp1.), four species were subdominant (*Nysius* sp., *Balclutha* sp2., *T. apicalis* and *Melanagromyza* sp.), three species were recedent (*H. phycitis*, *N. viridula* and *M. signata*) and all other 30 insect species recorded were subrecedent.

**CONCLUSION**

The study provides the first comprehensive documentation of insect pest diversity and abundance associated with carrots in India highlighting the urgent need for targeted pest management strategies, especially in the Eastern Dry Zone of Karnataka, to mitigate the impact of these pests on carrot crops and improve yield quality and

quantity. The study also contributes new host records of several insect species on carrots, marking a significant addition to the entomological literature in India.

#### ACKNOWLEDGEMENT

The authors are grateful to Dr. C. A. Viraktamath, Scientist Emeritus (Retd.), Department of Agricultural Entomology, University of Agricultural Sciences, GKVK, and Dr. H. M. Yeshwanth, Curator, Collection Facility at National Centre for Biological Sciences, Bengaluru, for insect identification.

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Received 24 September, 2024; Accepted 24 January, 2025



## Phytodiversity of Family Convolvulaceae in Bangladesh Agricultural University Campus

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**Abstract:** The Morning Glory family Convolvulaceae consists of about 60 genera with 2,000 species, mainly distributed in the tropics and subtropical regions of the world. A total of 22 taxa belonging to 6 genera are present at the Bangladesh Agricultural University (BAU) campus. With 14 species *Ipomoea* is the dominant genus followed by *Argyreia* and *Merremia* (2 species each). All taxa are important as medicine and food crops except for the well-known ornamental blue daze, *Evolvulus glomeratus* Nees & Mart. Moreover, *Ipomoea carnea* subsp. *fistulosa* has been identified as an invasive (alien) species for Bangladesh with minimal to moderate concern. The present communication provides a comprehensive description of members of the family Convolvulaceae at the BAU campus with colour photographs for easy identification.

**Keywords:** Convolvulaceae, *Ipomoea*, *Merremia*, Medicinal value

The cosmopolitan family Convolvulaceae, widespread across tropical and temperate regions, is one of the major families of flowering plants with approximately 2000 species, classified into 60 genera and 12 tribes (Staples and Brummitt 2007). Among the genera, *Ipomoea* and *Convolvulus* are the most species-rich genera of this family (Cronquist 1988). Members of the family Convolvulaceae are easily distinguishable by their plicate corolla, axile placentation with few ovules, bi-collateral vascular bundles and latex usually present. Important food crops like sweet potatoes and water spinach are included in the family, along with a variety of ornamental plants like morning glories and bindweeds, the parasitic vines Dodders, and medicinal uses for example fever, jaundice, boils and inflammation, epilepsy, central nervous system (CNS) disorders, etc. (Chen et al., 2018, Ashfaq et al., 2020). This family is represented in Bangladesh, by 15 genera and 55 species (Ahmed et al., 2008).

The Bangladesh Agricultural University (BAU) campus is located in Mymensingh district (Bangladesh) and is about 56.0 km away from Garo Hills (Fig. 1). It has an area of ca. 486 ha and the whole area can roughly be divided into three basic topographic types – plain, somewhat undulated, and basin-shaped low-lying areas, which gives a fairly wide range of habitats, viz. agricultural lands, fallow lands, ponds, marshy lands, gardens, grasslands, etc. for the growth of different types of plants. The soil type of the area varies from clay, and clay loam to sandy loam (UNDP 1988). The temperature ranges from 11.9 °C to 32.5 °C and the average annual rainfall is 244.15 mm. Most of the area is under cultivation for different seasonal crops and vegetables. Since

their commencement, BAU Botanical Garden (in 1963) and BAU Germplasm Centre (in 1991) have played an important role in collecting, curating, and managing forest and fruit tree germplasm from home and abroad. We have recently been trying to convert the whole university campus into a conservatory of agricultural (fruit) and non-agricultural (forest) plant genetic resources. Here, we provided a comprehensive description with colour photographs, common names, and uses, of members of the family Convolvulaceae at the BAU campus.

### MATERIAL AND METHODS

A detailed survey on the Family Convolvulaceae of the BAU campus has been carried out through frequent visits during 2020 - 2023. Plant samples were collected, curated, and conserved during these visits at the Botanical Garden, Department of Crop Botany, Bangladesh Agricultural University. The collected fresh (or dried) specimens were identified by comparing them with pre-identified herbarium specimens or pertinent published literature (Khan 1985, Ahmed et al., 2008, other online resources). The global distribution and botanical names were updated following the Plants of the World Online (<https://powo.science.kew.org/>), International Plant Names Index (<https://www.ipni.org/>), and World Flora Online Plant List (<https://wfoplantlist.org/plant-list>). Brief descriptions and colour photographs are provided for easy recognition of these plants.

Flowering samples of collected plants were processed into mounted herbarium specimens following standard procedure (Anonymous 1996). The mounted herbarium sheets are deposited in Prof. Arshad Ali Herbarium at the

Botanical Garden, Department of Crop Botany, Bangladesh Agricultural University (AAHBAU).

**RESULTS AND DISCUSSION**

A total of 22 species, mostly twinner, belonging to 6 genera are present at the BAU campus (Table 1). With 14 species *Ipomoea* is the most dominant genus followed by *Argyreia* and *Merremia* (2 species each). All the species have medicinal uses except *Evolvulus glomeratus* (Table 1). The oldest use for the family is possibly as a purgative or in religious ritual (Srivastava and Rauniyar 2020). Several pharmacological properties e.g., diuretic, anthelmintic, blood purifier, deobstruent, laxative, antimicrobial, analgesic, carminative and anti-inflammatory actions have been ascribed to these plants, besides their use to treat abdominal diseases, dysentery, fevers, headache, bronchitis, skin disorders, cervical lymphadenitis, fistulas, constipation, chronic gout, ulcers, haemorrhoids, tumours, obesity, jaundice, herpes, diabetes, hypertension, fatigue, arthritis, rheumatism, hydrocephaly, meningitis, kidney ailments, and induced lacrimation (Gupta and Ved 2017, Srivastava and Rauniyar 2020, Zia-UI-Haq et al., 2012), Japanese encephalitis (Srivastava and Shukla 2015), CNS disorders (Chen et al., 2018), sexually transmitted ailments, diabetes, rheumatism, cough, quinsy (Venkateswarlu and Ganapathy 2018), cancer (Jaseela et al., 2022), and many more.

Recently, *Ipomoea carnea* subsp. *fistulosa* has been identified as an invasive (alien) species in Bangladesh with

minimal to moderate concern (Anon. 2024). Therefore, proper care and management strategies should be taken to control the spread of this species, though it has medicinal uses (Table 1).

Taxa of the family Convolvulaceae observed at the BAU campus are briefly described below, including botanical names, synonyms, morphological features, representative specimens and worldwide distribution.

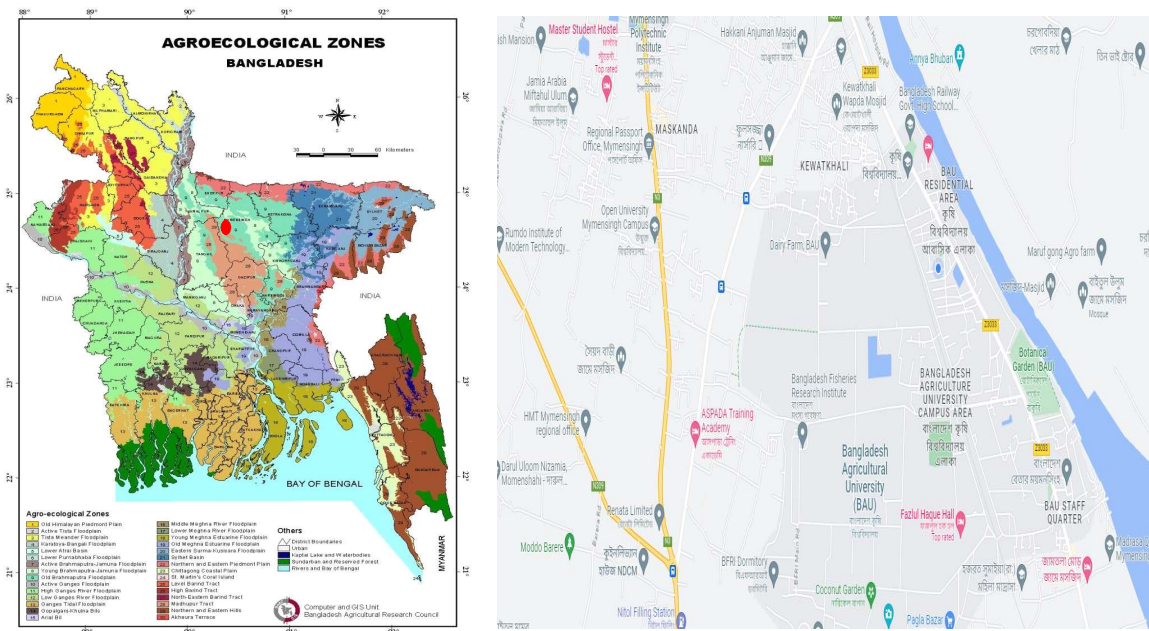
***Argyreia nervosa* (Burm. f.) Bojer Hortus Maurit.: 224. 1837.**

*Convolvulus nervosus* Burm. f., Fl. Ind.:48. 1768, Type: India, Fl. Ind. 1768. p. 48, tab. 20, fig. 1. *Convolvulus speciosus* L.f., Supp. Plant.:137. 1781. *Argyreiaspeciosa* (L.f.) Sweet, Sweet's Hort. Brit.:289. 1826. (Fig. 2A)

A woody perennial vine or liana, up to 15 m, young shoots and branches covered by a silky white pubescence. Leaves ovate-cordate, apiculate at tip, 15–25 cm x 13–20 cm, glabrous above, white tomentose (velvety) beneath, petiole white tomentose. Flowers pink or blue to purple with 7.5–30 cm long white-tomentose peduncles, borne in axillary many-flowered cymes; bracts ovate-lanceolate, acuminate, 3–4 cm long. Berry globose, 2 cm in diam., depressed apiculate, indehiscent, 4–6 seeds capsule<sup>-1</sup>.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 16 July 2020, Ashrafuzzaman & Sarwar 0005 (AAHBAU).

**Distribution:** Native to Bangladesh, India, Myanmar, Nepal; Introduced into Bermuda, Burkina, Cameroon, Central



**Fig. 1.** A. Geographic location of the study area Bangladesh Agricultural University campus (red dot in the Bangladesh Agroecological Zones map). B. Details of Bangladesh Agricultural University campus <<https://www.google.com/maps/@24.7255746,90.4219455,15z?entry=ttu>>

African Repu, Chad, Comoros, Cook Is., Cuba, Dominican Republic, Guinea, Haiti, Jamaica, Jawa, Leeward Is., Madagascar, Mauritius, Mozambique, New Caledonia, Nigeria, Pakistan, Panamá, Puerto Rico, Queensland, Rodrigues, Réunion, Senegal, Seychelles, Society Is., Sri Lanka, Sudan, Sumatera, Thailand, Tonga, USA, Venezuela, Windward Is., Zaire.

***Argyreiaroxburghii* (Sweet) Choisy Mém. Soc. Phys. Genève 6: 419. 1833 publ. 1834. [Conv. Or.: 37]**

*Ipomoea roxburghii* Sweet, Hort. Suburb. ed. 2, 289. 1826. *Argyreiaroxburghii* var. *siamica* Craib, Bull. Misc. Inform. Kew 1911. 423. 1911. (Fig. 2B)

A woody climber, stems to 4 m or taller, whitish hairy, glabrescent. Leaves ovate-cordate to suborbicular, 7–19 cm x 5.5–15.5 cm, acute softly patently villous on both surfaces, petiole 3–9 cm. Inflorescence erect or ascending, dichotomous, laxly cymose, 5 to many-flowered, peduncle 6–13 cm, flower funnellform, pale mauve, throat purple, midpetaline bands sparsely tomentose outside, otherwise

glabrous, 5–6.5 cm long. Berry globose, 13–15 mm, yellow or blackish, pulpy-soft, cupped by reflexed sepals. Seeds 4 capsule<sup>-1</sup>, subglobose.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 08 September 2023, Rahman, Sarwar & Ashrafuzzaman 001 (AAHBAU).

**Distribution:** Native to Bangladesh, Bhutan, East Himalaya, India, Myanmar, Nepal, Thailand; Introduced into Bermuda.

***Camoneaumbellata* (L.) A.R. Simões & Staples Bot. J. Linn. Soc. 183: 583. 2017.**

*Convolvulus umbellatus* L. Sp. Pl.:155. 1753. *Ipomoea polyanthes* Roem. & Schult. Syst. Veg., ed. 15[bis]. 4:234. 1819. *Merremiaumbellata* (L.) Hallier f. Bot. Jahrb. Syst. 16:552. 1893. (Fig. 2C)

A woody perennial twiner, slender with milky sap, up to 3 m in length. Leaves more or less cordate, 4–8 cm x 3–6 cm, acute at the apex, mucronulate, abaxially sparsely to densely hairy; hairs soft whitish, hairy or glabrescent adaxially; petiole 6–8 cm, glabrous or pubescent, a pair of auricles present at

**Table 1.** Members of the family Convolvulaceae collected from the Bangladesh Agricultural University campus.

Common/local name	Scientific name	Uses	Reference
Elephant creeper/Boro Dudhi	<i>Argyreia nervosa</i> (Burm. f.) Bojer	Weed, Medicinal	Grover 2021
Not Known	<i>Argyreia roxburghii</i> (Sweet) Choisy	Weed, Food, Medicinal	Barual et al., 2014
Yellow wood rose/Sada Kalmi	<i>Camonea umbellata</i> (L.) A.R. Simões & Staples	Vegetable, Medicinal	<a href="http://www.flowersofindia.net/">http://www.flowersofindia.net/</a>
Grape-leaf Wood Rose/Kornolata/ Halde provati	<i>Camonea vitifolia</i> (Burm.f.) A.R. Simões & Staples	Medicinal	Akter et al., 2021
Giant dodder/Shomolota	<i>Cuscuta reflexa</i> Roxb.	Medicinal	Muhammad et al., 2020
Blue daze	<i>Evolvulus glomeratus</i> Nees & Mart.	Ornamental	Srivastava & Rauniyar 2020
The Moon Flower/Dudhi Kalmi	<i>Ipomoea alba</i> L.	Vegetable, Weed, Feed, Medicinal	Rauniyar & Srivastava 2020
Water Spinach /Kalmi Shak	<i>Ipomoea aquatica</i> Forssk.	Vegetable, Feed, Medicinal	Alkiyumi et al., 2012
Sweet Potato/Misti Alu	<i>Ipomoea batatas</i> (L.) Lam.	Food, Feed, Medicinal	Mohanraj & Sivasankar 2014
Railway Creeper	<i>Ipomoea cairica</i> (L.) Sweet	Vegetable, Feed, Medicinal	Srivastava & Shukla 2015
Bush Morning Glory/Dhol kalmi	<i>Ipomoea carnea</i> Jacq.	Ornamental, Fuel, Food, Medicinal	Pant 2022
Pink Morning Glory	<i>Ipomoea carnea</i> subsp. <i>fistulosa</i> (Mart. ex Choisy) D.F. Austin	Medicinal	Mukherjee et al., 2011
Scarlet morning glory	<i>Ipomoea hederifolia</i> L.	Food, Medicinal	Zia-UI-Haq et al. 2012
Ocean Blue Morning Glory	<i>Ipomoea indica</i> (Burm.) Merr.	Ornamental, Fodder, Medicinal	Yuvarani & Selvam 2018
Palmate Morning Glory/Bhui Kumra	<i>Ipomoea mauritiana</i> Jacq.	Fodder, Medicinal, Food	Sulaiman et al., 2014
Beach Morning Glory/Chagol Kuri	<i>Ipomoea pes-caprae</i> (L.) R.Br.	Fodder, Medicinal	Akinniyi et al., 2022
Tiger's Footprint /Languli Lata	<i>Ipomoea pes-tigridis</i> L.	Medicinal	Selvam & Acharya 2015
Common morning-glory	<i>Ipomoea purpurea</i> (L.) Roth	Ornamental, Medicinal	Beheshti et al., 2021
Cypress Vine/Kunja Lata	<i>Ipomoea quamoclit</i> L.	Ornamental, Medicinal	Jaseela et al., 2022
Aiea morning glory/Choto ghanta	<i>Ipomoea triloba</i> L.	Medicinal, Food	Essiett & Obiobo 2014
Mexican morning glory/Indurkani	<i>Merremia emarginata</i> (Burm.f.) Hallier f.	Medicinal	Elumalai et al., 2011
Ivy Woodrose	<i>Merremia hederacea</i> (Burm.f.) Hallier f.	Feed, Medicinal	Charles et al., 2012.

base of petiole. Peduncles axillary, 10–12 cm, 8–10 flowered cymes, umbelliform. Pedicel 1–1.5 cm, flower dark yellow, funnel-shaped, 3–4 cm long, ca. 2.5–3 cm in breadth. Capsule sub-globose, ca. 1.5 x 1.2 cm in diameter, with ovate valves, brown coloured at maturity. Seeds 4 capsule<sup>-1</sup>, margins with longer brownish hairs.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 09 March 2021, Rahman, Sarwar & Ashrafuzzaman 008 (AAHBAU).

**Distribution:** Native to Argentina, Belize, Bolivia, Brazil, Burkina, Cameroon, Cayman Is., Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Florida, French Guiana, Gambia, Ghana, Guatemala, Guinea, Guyana, Haiti, Honduras, Ivory Coast, Jamaica, Leeward Is., Liberia, Mali, Mexico, Nicaragua, Nigeria, Panamá, Paraguay, Peru, Puerto Rico, Sierra Leone, Suriname, Trinidad-Tobago, Venezuela, Windward Is.; Introduced into Bangladesh, Comoros, Cook Is., East & West Himalaya, Galápagos, Hawaii, India, Jawa, Mauritius, Myanmar, Nepal, New Caledonia, Northern Territory, Queensland, Réunion, Samoa, Society Is., Sri Lanka, Tanzania, Vietnam.

**Camonea vitifolia (Burm.f.) A.R. Simões & Staples Bot. J. Linn. Soc. 183: 583. 2017.**

*Convolvulus vitifolius* Burm.f. Fl. Indica:45. 1768. *Ipomoea vitifolia* (Burm. f.) Sweet Hort. Brit.:289. 1826. nom. illeg. *Merremia vitifolia* (Burm. f.) Hallier f. Bot. Jahrb. Syst. 16:552. 1893. (Fig. 2D)

A twining shrub, stem brownish-black, young shoots patently hairy, up to 4 m. Leaves 5–7-lobed, 6–12 cm long, equally wide, base cordate, margin dentate-serrate, acute to acuminate at apex of lobation, sparsely fulvous hairy on both sides, basally 7-ribbed; petiole 3–8 cm. Inflorescence axillary, 1–3-flowered cymes; peduncles 4–5 cm, flower yellow, campanulate, 3.5–5 cm long, 4–6 cm across. Capsule 1–1.2 cm across, globose, fruiting sepals much larger. Seeds 4 capsule<sup>-1</sup>, ovoid.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 28 January 2023, Rahman, Sarwar & Ashrafuzzaman 001 (AAHBAU).

**Distribution:** Native to Andaman Is., Bangladesh, Cambodia, China, East Himalaya, India, Indonesia, Laos, Lesser Sunda Is., Malaya, Myanmar, Nepal, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam; Introduced into Cook Is.

**Cuscutareflexa Roxb. Pl. Coromandel 2: 3. 1799.**

*Kaduriasreflexa* (Roxb.) Raf. Fl. Tellur. 4:91. 1838. *Monogynellareflexa* (Roxb.) Holub Folia Geobot. Phytotax. 12:429. 1977. (Fig. 2E)

A stout succulent twiner, stem up to 2.5 mm in diam., yellowish. Flowers sessile in lateral racemes. Calyx cupular, lobes herbaceous, c. 1.5 mm long, suborbicular. Corolla

cream-coloured, c. 7 mm across, 6–8 mm long, funnel-form; lobes ovate-triangular, erect. Stamens 5; filaments very short; corolla scale ovate to oblong, fimbriate, ovary c. 2 mm long, conical, stigma acute. Capsule succulent, c. 5 mm across, globose-conical. Seeds black.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 10 January 2023, Rahman, Sarwar & Ashrafuzzaman 015 (AAHBAU).

**Distribution:** Native to Afghanistan, Bangladesh, China, East & West Himalaya, India, Indonesia, Laos, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, Tibet, Vietnam; Introduced into Mauritius.

**Evolvulus glomeratus Nees & Mart. Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur. 11: 81. 1823.**

*Evolvulus glomeratus* subsp. *euglomeratus* Ooststr. Meded. Bot. Mus. Herb. Rijks Univ. Utrecht 14:225. 1934, not validly publ. *Evolvulus glomeratus* var. *genuinus* Meisn. C.F.P. von Martius & auct. suc. (eds.), Fl. Bras. 7:335. 1869, not validly publ. *Evolvulus glomeratus* f. *genuinus* Ooststr. Meded. Bot. Mus. Herb. Rijks Univ. Utrecht 14:226. 1934. (Fig. 2F)

An evergreen woody subshrub, up to 0.4 m tall and 1 m in diameter. Leaves and stems densely downy, covered with a light grey fuzz, lanceolate to elliptic, 0.8–1.5 cm x 2–3 cm. Flowers solitary, plate to funnel-shaped, up to 2 cm across, pale lavender or powder blue petals and white throats.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 15 May 2023, Rahman, Sarwar & Ashrafuzzaman 019 (AAHBAU).

**Distribution:** Native to Argentina, Bolivia, Brazil, French Guiana, Guyana, Paraguay, Suriname, Uruguay, Venezuela; Introduced into Bangladesh, Leeward Is., Windward Is.

**Ipomoea alba L. Sp. Pl. 161. 1753.**

*Ipomoea bona-nox* L. Sp. Pl., ed. 2:228. 1762, nom. superfl. *Ipomoea grandiflora* Lam. Tabl. Encycl. 1:467. 1793. *Convolvulus bona-nox* Spreng. Syst. Veg., ed. 16. 1:600. 1824. *Calonyction roxburghii* G. Don in Gen. Hist. 4:263. 1837. *Calonyction album* (L.) House Bull. Torrey Bot. Club 31:591. 1904. (Fig. 2G)

A perennial climber, the stem woody at the base. Leaves rounded, ovate, entire or 3–5-lobed, 5–15 cm long, basally cordate, apically acuminate, glabrous. Inflorescence 1 to several flowered cymes, flowers white, salverform, the tube 9–15 cm long, the limb 8–10 cm broad. Fruit capsular, ovoid to subglobose, 2–3 cm long, dark brown, glabrous. Seeds dark brown to black, glabrous.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 08 February 2023, Rahman, Sarwar & Ashrafuzzaman 009 (AAHBAU).

**Distribution:** Native to Argentina, Bahamas, Belize, Bolivia, Brazil, Central American Pac, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Florida, French Guiana, Galápagos, Guatemala, Guyana, Haiti, Honduras, Jamaica, Leeward Is., Mexico, Nicaragua, Panamá, Paraguay, Peru, Puerto Rico, Suriname, Trinidad-Tobago, Uruguay, Venezuela, Windward Is.; Introduced into Andaman Is., Angola, Australia, Bangladesh, Bermuda, Cameroon, Caroline Is., Central African Repu, China, Colorado, Comoros, Congo, Cook Is., East & West Himalaya, Equatorial Guinea, Ethiopia, Fiji, Gabon, Ghana, Guinea, Gulf of Guinea Is., India, Ivory Coast, Japan, Kenya, Indonesia, Kermadec Is., Korea, Laos, Lesser Sunda Is., Liberia, Line Is., Madagascar, Malaya, Marianas, Marquesas, Mauritius, Mozambique, Myanmar, Nepal, New Caledonia, New Guinea, Nigeria, Norfolk Is., Pakistan, Philippines, Pitcairn Is., Réunion, South Africa, Samoa, Sierra Leone, Society Is., Solomon Is., Sri Lanka, St. Helena, Sudan, Sulawesi, Tanzania, Texas, Thailand, Togo, Tokelau-Manihiki, Tonga, Tuamotu, Tubuai Is., Uganda, USA, Vanuatu, Vietnam, Zaïre, Zimbabwe.

***Ipomoea aquatica* Forssk. Fl. Aegypt.-Arab.: 44. 1775.** (Fig. 2H)

A creeping or floating aquatic herb, stems hollow, rooting at the nodes. Leaves alternate, usually oblong-lanceolate or narrowly triangular, 5–10 cm x 2–6 cm, base hastate, apex acute; petiole 6–10 cm long. Flowers purplish-white, solitary or few in cymes, funnel-form, c. 5 cm long, pale purple to nearly white, tube to 2 cm long, lobes obscure. Capsule globose, seeds 4 or 2 capsule<sup>-1</sup>, minutely pubescent.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 21 October 2021, Rahman, Sarwar & Ashrafuzzaman 001 (AAHBAU).

**Distribution:** Native to Angola, Bangladesh, Borneo, Botswana, Burundi, Cambodia, Cameroon, Caprivi Strip, Caroline Is., Central African Repu, Chad, China, Comoros, Congo, Eritrea, Ethiopia, Fiji, Gabon, Gambia, Ghana, Gilbert Is., Guinea, Gulf States, India, Ivory Coast, Jawa, Kenya, KwaZulu-Natal, Laos, Lesser Sunda Is., Liberia, Madagascar, Malawi, Malaya, Mali, Maluku, Marianas, Mauritania, Mozambique, Myanmar, Namibia, Nepal, New Guinea, Nigeria, Northern Territory, Oman, Pakistan, Philippines, Queensland, Saudi Arabia, Senegal, Sierra Leone, Somalia, Sri Lanka, Sudan, Sulawesi, Sumatera, Tanzania, Thailand, Togo, Uganda, Vanuatu, Vietnam, West Himalaya, Western Australia, Yemen, Zambia, Zaïre, Zimbabwe; Introduced into Aruba, Belize, Brazil, Burkina, Christmas Is., Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Florida, French Guiana, Guatemala, Guyana, Haiti, Hawaii, Honduras, Jamaica, Leeward Is.,

Mauritius, Mississippi, Nauru, New Caledonia, Nicaragua, Palestine, Panamá, Peru, Puerto Rico, Réunion, Samoa, Seychelles, Society Is., Solomon Is., South China Sea, Suriname, Trinidad-Tobago, Wallis-Futuna Is.

***Ipomoea batatas* (L.) Lam. Tabl. Encycl. 1: 465. 1793.**

*Convolvulus batatas* L. Sp. Pl.:154. 1753. *Convolvulus esculentus* Salisb. Prodr. Stirp. Chap. Allerton: 123. 1796, nom. superfl. *Solanum batatas* (L.) Aikman in Barham, Hort. Amer.: 222, 153. 1794. (Fig. 2I)

A creeping perennial vine with tuberous roots, up to 4 m long. Leaf shape ranges from ovate-cordate to palmate, spirally arranged, petiole long, margin entire or palmately lobed, 5–10 cm long. Flowers axillary solitary or inflorescence few-flowered cyme, white or purplish, funnel-shaped, 4–7 cm long. Capsule 5–8 mm long, containing 1–4 seeds capsule<sup>-1</sup>, black and very hard seed coats.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 07 October 2023, Rahman, Sarwar & Ashrafuzzaman 025 (AAHBAU).

**Distribution:** Native to Belize, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panamá, Venezuela; Introduced into Algeria, Angola, Argentina, Azores, Bahamas, Bangladesh, Bermuda, Bolivia, Borneo, Brazil, Burkina, Burundi, Cambodia, Cameroon, Canary Is., Cape Provinces, Cape Verde, Caroline Is., Cayman Is., Central African Repu, Chad, Chile North, China, Christmas I., Comoros, Congo, Cook Is., Cuba, Dominican Republic, East Himalaya, Easter Is., Egypt, Eritrea, Ethiopia, Fiji, French Guiana, Galápagos, Ghana, Gilbert Is., Greece, Guinea, Guinea-Bissau, Guyana, Haiti, India, Ivory Coast, Jamaica, Jawa, Kenya, Kermadec Is., Kirgizstan, Korea, KwaZulu-Natal, Laos, Leeward Is., Lesser Sunda Is., Libya, Line Is., Madagascar, Madeira, Malawi, Malaya, Maluku, Marianas, Marquesas, Marshall Is., Mauritania, Mauritius, Mexico, Morocco, Mozambique, Myanmar, Nansei-shoto, Nauru, Nepal, New Caledonia, New Guinea, New York, New Zealand North, Nigeria, Niue, Pakistan, Paraguay, Peru, Philippines, Portugal, Puerto Rico, Queensland, Rodrigues, Rwanda, Réunion, Saudi Arabia, Senegal, Seychelles, Sierra Leone, Society Is., Solomon Is., Somalia, Spain, Sri Lanka, St. Helena, Sudan, Sulawesi, Sumatera, Suriname, Tadzhikistan, Tanzania, Thailand, Tokelau-Manihiki, Tonga, Transcaucasus, Trinidad-Tobago, Tuamotu, Tubuai Is., Turkmenistan, Tuvalu, Uganda, Uruguay, USA, Vanuatu, Venezuelan Antilles, Vietnam, Wake I., Western Australia, Windward Is., Yemen, Zaïre, Zimbabwe.

***Ipomoea cairica* (L.) Sweet Hort. Brit.: 287. 1826.**

*Convolvulus cairicus* L. Syst. Nat., ed. 10. 2:922. 1759.

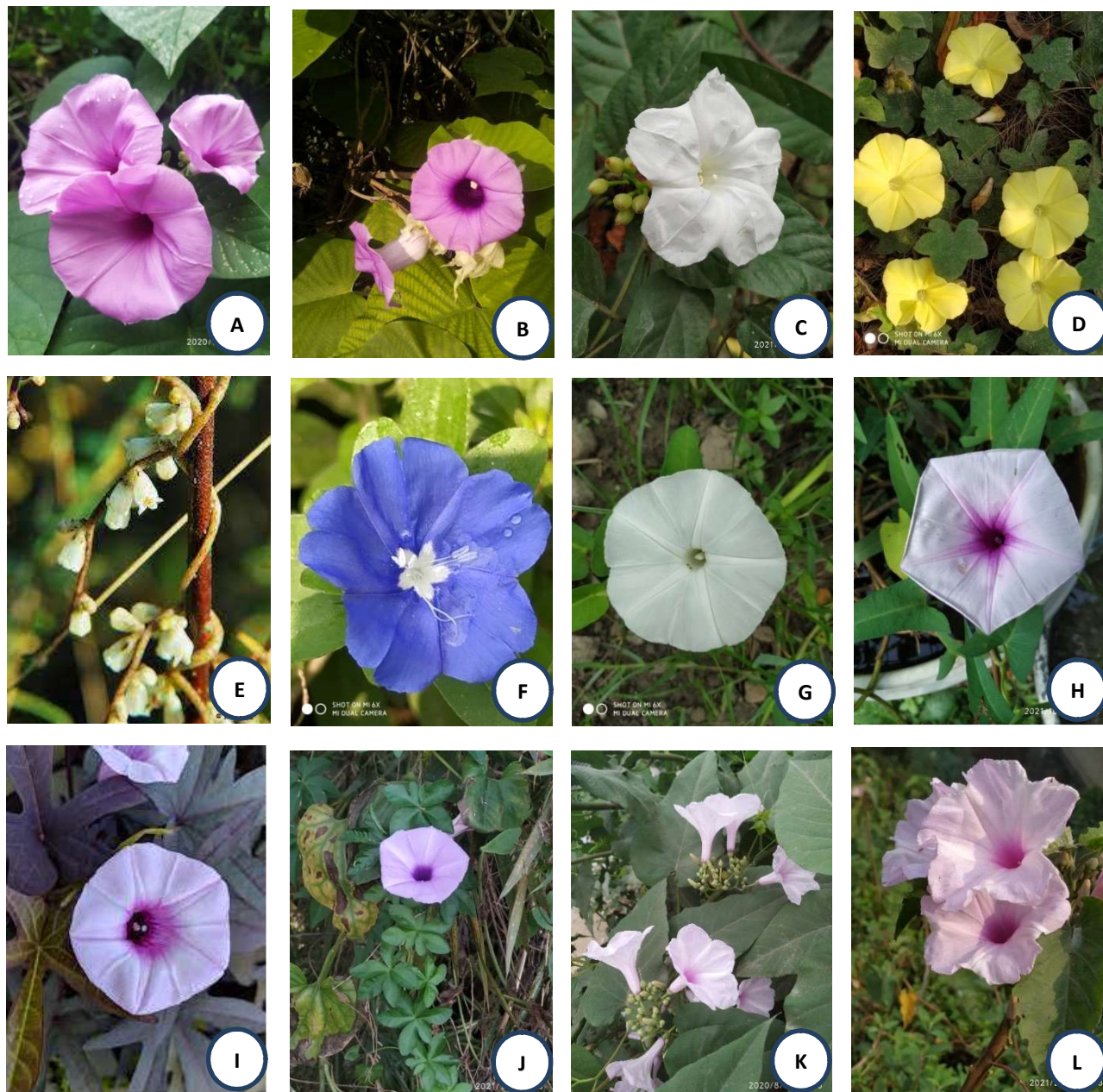
*Exocroaegyptiaca* Raf. Fl. Tellur. 4:80. 1838, nom. superfl. (Fig. 2J)

A perennial climbing plant, up to 5 m, somewhat woody at its base. Petiole 2-8 cm, base with leafy pseudostipules, leaf blade palmately 5-parted to base; apex acute or obtuse, mucronulate, ovate, ovate-lanceolate, or elliptic, (2.5–)4–5 X (0.5–)2–2.5 cm. Inflorescences 1- or several flowered, peduncle 2-8 cm, pedicel 0.5-2 cm, outer 2 sepals 4–6.5 mm; inner ones 5–9 mm, glabrous, corolla pink, purple, or reddish purple, with a darker centre, rarely white, funnellform, (2.5–)

5–7 cm. Capsule globose, ca. 1 cm. Seeds black, densely tomentose.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 03 January 2021, Rahman, Sarwar & Ashrafuzzaman 017 (AAHBAU).

**Distribution:** Native to Angola, Bangladesh, Botswana, Burkina, Burundi, Cameroon, Cape Provinces, China, Congo, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Gulf of Guinea Is., Hainan, India, Ivory Coast, Japan, Kenya, KwaZulu-Natal,



**Fig. 2.** Photographs of different Convolvulaceae species. (A) *Argyreia nervosa*, (B) *Argyreiaroxburghii*, (C) *Camoneaumbellata*, (D) *Camoneavitifolia*, (E) *Cuscutareflexa*, (F) *Evolvulusglomeratus*, (G) *Ipomoea alba*, (H) *Ipomoea aquatica*, (I) *Ipomoea batatas*, (J) *Ipomoea cairica*, (K) *Ipomoea carnea*, and (L) *Ipomoea carneasubsp. fistulosa*



Laos, Liberia, Madagascar, Malawi, Mauritius, Mozambique, Myanmar, Nepal, Nigeria, Northern Provinces, Oman, Palestine, Rodrigues, Rwanda, Réunion, Senegal, Sierra Leone, Somalia, South China Sea, Sri Lanka, Sudan, Swaziland, Taiwan, Tanzania, Thailand, Togo, Uganda, Vietnam, West Himalaya, Yemen, Zambia, Zaïre, Zimbabwe; Introduced into Argentina, Assam, Australia, Bismarck Archipelago, Bolivia, Borneo, Brazil, California, Canary Is., Cape Verde, Cayman Is., Christmas I., Colombia, Cuba, Ecuador, Egypt, Fiji, Guyana, Jamaica, Jawa, Kermadec Is., Leeward Is., Malaya, Maluku, Mexico, New Caledonia, New Guinea, New Zealand, Niue, Norfolk Is., Pakistan, Paraguay, Peru, Philippines, Saudi Arabia, Seychelles, Sicilia, Socotra, Tonga, Trinidad-Tobago, Tubuai Is., Uruguay, USA, Vanuatu, Venezuela, Western Australia, Windward Is.

***Ipomoea carnea* Jacq. Enum. Syst. Pl.: 13. 1760.**

*Convolvulus carneus* (Jacq.) Spreng. Syst. Veg., ed. 16. 1:602. 1824. (Fig. 2K)

A shrub, up to 5 m tall. Leaves suborbicular or ovate to lanceolate, up to 30 cm long, apex acuminate, slightly cordate leaf base, pubescent especially beneath. Flowers are arranged in cymes/panicles, dark pink to purple, slightly enlarged at the base. Capsule ovoid, glabrous. Seed many, black, pubescent.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 31 August 2020, Ashrafuzzaman, Sarwar & Rahman 0096 (AAHBAU).

**Distribution:** Native to Argentina, Belize, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panamá, Paraguay, Peru, Venezuela; Introduced into Aruba, Australia, Bahamas, Bangladesh, Burkina, Burundi, Cambodia, Cameroon, Caroline Is., Chad, China Southeast, Colorado, Comoros, Cuba, Dominican Republic, East & West Himalaya, Egypt, Eritrea, Ethiopia, Fiji, Guinea, Hainan, Haiti, India, Iran, Ivory Coast, Jamaica, Jawa, Kenya, KwaZulu-Natal, Laos, Leeward Is., Lesser Sunda Is., Libya, Madagascar, Malawi, Malaya, Marianas, Marquesas, Mauritius, Mozambique, Myanmar, Nansei-shoto, Nauru, Nepal, Netherlands Antilles, New Caledonia, New Guinea, Pakistan, Puerto Rico, Rodrigues, Rwanda, Réunion, Saudi Arabia, Seychelles, Society Is., Somalia, Sri Lanka, Sudan, Taiwan, Tanzania, Thailand, Trinidad-Tobago, Venezuelan Antilles, USA, Vietnam, Windward Is., Yemen, Zambia, Zaïre, Zimbabwe.

***Ipomoea carnea* subsp. *fistulosa* (Mart. ex Choisy) D.F.Austin Taxon 26: 237. 1977.**

*Convolvulus fistulosus* (Mart. ex Choisy) Kuntze in Revis. Gen. Pl. 3(2): 213. 1898. *Ipomoea fistulosa* Mart. ex Choisy in A.P.de Candolle, Prodr. 9:349. 1845. (Fig. 2L)

A shrub, woody at the base, hollow, glabrous, or minutely

puberulent, up to 2.5 m. Leaves ovate to lanceolate, 10–25 cm long, truncate to shallowly cordate basally, long acuminate apically, puberulent on both surfaces. Flowers in cymose-paniculate clusters, deep pink to rose-purple to almost white, 5–8 cm long. Fruit capsular, ovoid to subglobose, 2 cm long. Seeds covered with long comose brown trichomes.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 17 February 2021, Rahman, Sarwar & Ashrafuzzaman 022 (AAHBAU).

**Distribution:** Native to Argentina, Belize, Bolivia, Brazil, North, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panamá, Paraguay, Peru, Venezuela; Introduced into Australia, Bahamas, Bangladesh, Burundi, Cambodia, Cameroon, Caroline Is., Chad, China Southeast, Colorado, Comoros, Cuba, Dominican Republic, East & West Himalaya, Eritrea, Ethiopia, Fiji, Florida, Georgia, Guinea, Hainan, Haiti, India, Iran, Ivory Coast, Jamaica, Jawa, Kenya, KwaZulu-Natal, Laos, Leeward Is., Lesser Sunda Is., Madagascar, Malawi, Malaya, Marianas, Marquesas, Mauritius, Mozambique, Myanmar, Nansei-shoto, Nauru, Nepal, Netherlands Antilles, New Caledonia, New Guinea, Pakistan, Puerto Rico, Rodrigues, Rwanda, Réunion, Saudi Arabia, Seychelles, Society Is., Somalia, South Carolina, Sri Lanka, Sudan, Taiwan, Tanzania, Texas, Thailand, Trinidad-Tobago, Vietnam, Windward Is., Zambia, Zaïre, Zimbabwe.

***Ipomoea hederifolia* L. Syst. Nat., ed. 10. 2: 925. 1759.**

*Convolvulus coccineus* var. *hederifolius* (L.) Kuntze in Revis. Gen. Pl. 3(2):213. 1898. *Ipomoea coccinea* var. *hederifolia* (L.) A.Gray Syn. Fl. N. Amer. 2(1):209. 1878. *Mina hederifolia* (L.) Bello Apuntes Fl. Puerto-Rico 1:294. 1881. *Quamoclit coccinea* var. *hederifolia* (L.) House Ann. New York Acad. Sci. 18:262. 1908. *Quamoclit hederifolia* (L.) G.Don Gen. Hist. 4:259. 1837. (Fig. 3A)

An annual climber, glabrous to sparsely pubescent. Leaves ovate to suborbicular, 2–15 cm long, entire, dentate, trilobate or with 5–7 lobes, basally cordate, acute to acuminate apically, mostly glabrous. Flowers in few-to several-flowered cymes or solitary, red to red-yellow, 2.5–4.5 cm long, salverform. Fruit capsular, subglobose, 6–8 mm long. Seeds dark brown or black, pyriform.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 08 May 2023, Rahman, Sarwar & Ashrafuzzaman 027 (AAHBAU).

**Distribution:** Native to Argentina, Belize, Bermuda, Bolivia, Brazil, Cayman Is., Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Florida, French Guiana, Guatemala, Guyana, Haiti, Honduras, Jamaica, Leeward Is., Mexico, Nicaragua, Panamá, Paraguay, Peru, Puerto Rico,

Suriname, Trinidad-Tobago, USA, Venezuela, Windward Is.; Introduced into Angola, Australia, Bahamas, Bangladesh, Burundi, Cameroon, Central African Repu, China, Christmas I., Comoros, East & West Himalaya, Equatorial Guinea, Fiji, Ghana, Gulf of Guinea Is., Hawaii, India, Ivory Coast, Kenya, Korea, Madagascar, Malawi, Malaya, Marianas, Mauritius, Mozambique, Myanmar, Nauru, Nepal, New Caledonia, Nigeria, North Carolina, Northern Territory, Pakistan, Primorye, Rodrigues, Réunion, Senegal, Seychelles, Sierra Leone, Sri Lanka, Sudan, Tanzania, Thailand, Togo, Tonga, Tubuai Is., Uganda, Vanuatu, Vermont, Vietnam, Wallis-Futuna Is., Zambia, Zaïre, Zimbabwe.

***Ipomoea indica* (Burm.) Merr. Interpr. Herb. Amboin.: 445. 1917.**

*Convolvulus indicus* Burm. Auctuarium: 2 verso. 1755. *Pharbitis indica* (Burm.) Hagiw. Bot. & Zool. 6:1238. 1938. (Fig. 3B)

Atwining herb, sometimes prostrate, up to 6 m. Leaf blade ovate or circular, 5–15 cm x 3.5–14 cm, abaxially densely pubescent, adaxially sparsely pubescent, base cordate, apex acuminate or abruptly acuminate, petiole 2–18 cm. Inflorescences dense umbellate cymes, several flowered; peduncle 4–20 cm. Pedicel 2–5(–8) mm, flowers bright blue or bluish purple, ageing reddish-purple or red, with a paler centre, funnellform, 5–8 cm, glabrous. Capsule globose, 1–1.3 cm in diam.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 12 May 2019, Ashrafuzzaman & Sarwar 0105 (AAHBAU).

**Distribution:** Native to Argentina, Bahamas, Belize, Brazil, Cayman Is., Central American Pac, Colombia, Costa Rica, Cuba, Dominican Republic, Florida, French Guiana, Guatemala, Guyana, Haiti, Honduras, Jamaica, Leeward Is., Mexican Pacific Is., Mexico, Nicaragua, Panamá, Paraguay, Puerto Rico, Suriname, Texas, Trinidad-Tobago, Uruguay, Venezuela, Venezuelan Antilles, Windward Is.; Introduced into Alabama, Algeria, Assam, Australia, Azores, Baleares, Bangladesh, Bermuda, Bolivia, California, Cameroon, Canary Is., Cape Provinces, Caroline Is., Chile Central, China Southeast, Comoros, Congo, Cook Is., East Aegean Is., East Himalaya, Eritrea, Ethiopia, Fiji, France, Gabon, Galápagos, Ghana, Greece, Guinea, Hawaii, Japan, Juan Fernández Is., Kazan-retto, Kenya, Kriti, KwaZulu-Natal, Laos, Lesser Sunda Is., Madagascar, Madeira, Malawi, Malaya, Maluku, Marianas, Mauritius, Morocco, Myanmar, Nepal, New Caledonia, New Guinea, New Zealand, Nigeria, Niue, Norfolk Is., Northern Provinces, Pakistan, Palestine, Peru, Philippines, Pitcairn Is., Portugal, Rodrigues, Rwanda, Réunion, Samoa, Senegal, Sicilia, Sierra Leone, Society Is., Solomon Is., Somalia, South China Sea, Spain, Sri Lanka,

Sulawesi, Sumatera, Swaziland, Taiwan, Tanzania, Tasmania, Tonga, Tuamotu, Tubuai Is., Tunisia, Vanuatu, Victoria, Vietnam, Yugoslavia, Zambia, Zaïre, Zimbabwe.

***Ipomoea mauritiana* Jacq. Collectanea 4: 216. 1791.**

*Ipomoea paniculata* var. *mauritiana* (Jacq.) Kuntze in Revis. Gen. Pl. 2:445. 1891. (Fig. 3C)

Aperennial twining herb, to 10 m. Leaf circular, 7–18 cm X 7–22 cm, palmately 5–7 lobes, segments lanceolate or elliptic, apex acuminate or acute, mucronulate, petiole 3–11 cm. Inflorescences few to many flowered, peduncle 2.5–20 cm. Pedicel 0.9–2.2 cm, flowers pink or reddish purple, with a darker centre, funnellform, 5–6 cm. Capsule ovoid, 1.2–1.4 cm. Seeds dark brown, woolly-sericeous with long, easily detached hairs.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 08 February 2023, Rahman, Sarwar & Ashrafuzzaman 028 (AAHBAU).

**Distribution:** Native to Angola, Belize, Benin, Bolivia, Brazil, Burkina, Burundi, Cameroon, Cape Verde, Central African Repu, Chad, Colombia, Comoros, Congo, Costa Rica, Dominican Republic, Ecuador, Equatorial Guinea, French Guiana, Gabon, Gambia, Ghana, Guatemala, Guinea, Guinea-Bissau, Gulf of Guinea Is., Guyana, Haiti, Honduras, Ivory Coast, Jamaica, Kenya, KwaZulu-Natal, Liberia, Madagascar, Malawi, Mauritius, Mozambique, Nicaragua, Nigeria, Panamá, Peru, Puerto Rico, Rodrigues, Réunion, Senegal, Sierra Leone, Somalia, Sudan, Suriname, Tanzania, Togo, Uganda, Venezuela, Zambia, Zaïre, Zimbabwe; Introduced into Bangladesh, Cambodia, Caroline Is., China, Christmas I., Fiji, India, Kazan-retto, Laos, Leeward Is., Lesser Sunda Is., Malaya, Myanmar, Nansai-shoto, Nepal, New Caledonia, New Guinea, Northern Territory, Ogasawara-shoto, Philippines, Queensland, Seychelles, Sri Lanka, Sulawesi, Taiwan, Thailand, Trinidad-Tobago, Vietnam, Windward Is.

***Ipomoea caprae* (L.) R.Br. J.H.Tuckey, Narr. Exped. Zaire: 477. 1818.**

*Convolvulus caprae* L. Sp. Pl.:159. 1753. *Convolvulus capripes* Stokes Bot. Mat. Med. 1:327. 1812, nom. superfl. *Ipomoea aegopoda* St.-Lag. Ann. Soc. Linn. Lyon 7:70. 1880, nom. superfl. *Plesiagopussovana* Raf. Fl. Tellur. 4:78. 1838, nom. superfl. (Fig. 3D)

A perennial herb, prostrate, up to 30 m, rooting at nodes. Petiole 2–10 cm, leaf ovate, elliptic, circular, reniform or quadrate to oblong, 3.5–9 cm X 3–10 cm, base broadly cuneate, truncate, or shallowly cordate, apex emarginate or deeply 2-lobed, mucronulate. Inflorescences 1- to several flowered, peduncle 4–14 cm. Pedicel 2–2.5 cm, flower purple or reddish purple, with a darker center, funnellform, 4–5 cm. Capsule globular, 1.1–1.7 cm, leathery, seeds black,

trigonus-globose, densely brownish tomentose.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 20 November 2020, Ashrafuzzaman & Sarwar 0041 (AAHBAU).

**Distribution:** Native to Aldabra, Angola, Aruba, Australia, Bahamas, Bangladesh, Belize, Benin, Bermuda, Borneo, Brazil, Burundi, Cambodia, Cameroon, Canary Is., Cape Provinces, Cape Verde, Caroline Is., Cayman Is., Central American Pac, China Southeast, Christmas I., Cocos (Keeling) Is., Colombia, Comoros, Congo, Cook Is., Costa Rica, Cuba, Djibouti, Dominican Republic, Easter Is., Ecuador, Egypt, El Salvador, Equatorial Guinea, Eritrea, Fiji, French Guiana, Gabon, Galápagos, Georgia, Ghana, Gilbert Is., Guatemala, Guinea, Gulf of Guinea Is., Gulf States, Guyana, Hainan, Haiti, Hawaii, Honduras, India, Iran, Ivory Coast, Jamaica, Japan, Jawa, Kazan-retto, Kenya, Kermadec Is., Kuwait, KwaZulu-Natal, Leeward Is., Lesser Sunda Is., Liberia, Line Is., Louisiana, Madagascar, Madeira, Malawi, Malaya, Maluku, Marcus I., Marianas, Marquesas, Marshall Is., Mauritania, Mauritius, Mexico, Mississippi, Mozambique, Myanmar, Nansei-shoto, Nauru, Netherlands Antilles, New Caledonia, New Guinea, New Zealand North, Nicaragua, Nigeria, Norfolk Is., Northern Territory, Ogasawara-shoto, Oman, Pakistan, Palestine, Panamá, Peru, Philippines, Phoenix Is., Puerto Rico, Rodrigues, Réunion, Samoa, Saudi Arabia, Senegal, Seychelles, Sierra Leone, Sinai, Society Is., Socotra, Solomon Is., Somalia, South China Sea, Sri Lanka, Sudan, Sulawesi, Sumatera, Suriname, Taiwan, Tanzania, Thailand, Togo, Tokelau-Manihiki, Tonga, Trinidad-Tobago, Tuamotu, Tubuai Is., Turks-Caicos Is., Tuvalu, USA, Vanuatu, Venezuela, Venezuelan Antilles, Vietnam, Wallis-Futuna Is., Windward Is., Yemen, Zambia, Zaïre, Zimbabwe; Introduced into Wake I.

***Ipomoea pes-tigridis* L. Sp. Pl.: 162. (1753) nom. cons.**

*Convolvuloides palmata* Moench Methodus:452. 1794, nom. superfl. *Convolvulus bryoniifolius* Salisb. Prodr. Stirp. Chap. Allerton:125. 1796, nom. superfl. *Convolvulus pes-tigridis* (L.) Spreng. Syst. Veg., ed. 16. 1:502. 1824. (Fig. 3E)

A twining annual herb, up to 3 m. Leaf circular or transversely elliptic, 2–10 cm x 3–13 cm, densely pubescent, apex mucronate, petiole 2–8 cm. Inflorescences capitate, few-flowered, peduncle 4–11 cm, pedicel obsolete. Corolla white, funnelform, 3–4 cm, midpetaline bands sparsely pubescent. Capsule ovoid, ca. 7 mm, 4-valved. Seeds ellipsoid, grey tomentellous.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 08 October 2023, Rahman, Sarwar & Ashrafuzzaman 027 (AAHBAU).

**Distribution:** Native to Angola, Bangladesh, Benin, Borneo,

Botswana, Burkina, Cambodia, Caprivi Strip, Central African Repu, Chad, China, East Himalaya, Guinea-Bissau, Hainan, India, Jawa, Kenya, Lesser Sunda Is., Malawi, Malaya, Mali, Maluku, Mauritius, Mozambique, Myanmar, Namibia, Nepal, New Guinea, Niger, Nigeria, Northern Provinces, Pakistan, Philippines, Somalia, South China Sea, Sri Lanka, Sudan, Sulawesi, Sumatera, Taiwan, Tanzania, Thailand, Vietnam, West Himalaya, Zambia, Zaïre, Zimbabwe; Introduced into Australia, Marianas, Northern Territory.

***Ipomoea purpurea* (L.) Roth Bot. Abh. Beobacht.: 27. 1787.**

*Convolvulus purpureus* L. Sp. Pl., ed. 2.:219. 1762. *Convolvuloides purpurea* (L.) Moench Methodus:452. 1794. *Diatremapurpurea* (L.) Raf. Fl. Tellur. 4:72. 1838. *Pharbitis purpurea* (L.) Bojer Hortus Maurit.:227. 1837. (Fig. 3F)

An annual climber, pilose to hirsute with spreading trichomes. Leaves broadly ovate to cordate, 2–10 cm long, entire or trilobate, pubescent on both surfaces. Inflorescence 1–5-flowered cymes, flower purple, pink, blue or with stripes of these colours on a white background, throat white, 3–5 cm long. Fruit capsular, depressed globose, 10 mm long. Seeds black, pyriform, glabrous.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 08 September 2023, Rahman, Sarwar & Ashrafuzzaman 031 (AAHBAU).

**Distribution:** Native to Argentina, Bolivia, Brazil, Chile Central, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Jamaica, Mexico, Nicaragua, Panamá, Paraguay, Peru, Uruguay, USA, Venezuela; Introduced into Angola, Australia, Austria, Balears, Bangladesh, British Columbia, Bulgaria, Canada, Canary Is., Cape Provinces, Cape Verde, Central European Rus, China, Cuba, Cyprus, Dominican Republic, East European Russia, East & West Himalaya, Egypt, Eritrea, Ethiopia, France, Free State, Great Britain, Greece, Hainan, Haiti, India, Iran, Italy, Kenya, Korea, KwaZulu-Natal, Leeward Is., Lesotho, Madagascar, Madeira, Maryland, Mauritius, Michigan, Mozambique, Myanmar, Nepal, New Caledonia, New Zealand, Northern Provinces, Pakistan, Philippines, Primorye, Puerto Rico, Rodrigues, Romania, Rwanda, Réunion, Sicilia, South European Russi, Spain, Sri Lanka, Swaziland, Tadzhikistan, Tanzania, Thailand, Tibet, Tunisia, Turkey, Uganda, Windward Is., Yemen, Zambia, Zaïre, Zimbabwe.

***Ipomoea quamoclit* L. Sp. Pl.: 159. 1753.**

*Convolvulus pennatifolius* Salisb. Prodr. Stirp. Chap. Allerton: 124. 1796, nom. superfl. *Convolvulus pennatus* Desr. in J.B.A.M.de Lamarck, Encycl. 3:567. 1792, nom. superfl. *Convolvulus quamoclit* (L.) Spreng. Syst. Veg., ed. 16. 1:591. 1824. *Quamoclit pennata* Bojer Hort. Maurit.:224.

1837, nom. superfl. *Quamoclit quamoclit* (L.) Britton in N.L.Britton&A.Brown, III. Fl. N. U.S. 3:22. 1898, not validly publ. *Quamoclit vulgaris* Choisy yMém. Soc. Phys. Genève 6:434. 1833 publ. 1834 [Conv. Or.: 52]. (Fig. 3G)

An herbaceous, twining vine, up to 3.0 m tall. Leaf deeply lobed (nearly pinnate), 9–19 lobes on each side, 1–9 cm long. Flowers solitary or in 2-5-flowered cymes, red, pink, or white, trumpet-shaped, 2.5–5.1 cm x 2.5 cm. Fruit capsular, ovoid, 6–8 mm long. Seeds 4 capsule<sup>-1</sup>, ovoid, black.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 20 September 2019, Ashrafuzzaman& Sarwar 0071 (AAHBAU).

**Distribution:** Native to Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panamá; Introduced into Angola, Argentina, Australia, Bahamas, Bangladesh, Benin, Bermuda, Bolivia, Borneo, Brazil, Burundi, Cambodia, Cameroon, Cape Verde, Caroline Is., Cayman Is., Central African Repu, Chad, China Southeast, Christmas Is., Colombia, Comoros, Cuba, Dominican Republic, East & West Himalaya, Ecuador, Equatorial Guinea, Fiji, French Guiana, Gabon, Galápagos, Gambia, Georgia, Guinea, Gulf of Guinea Is., Guyana, Haiti, India, Ivory Coast, Jamaica, Korea, Laos, Leeward Is., Lesser Sunda Is., Liberia, Madagascar, Madeira, Malawi, Malaya, Marianas, Marquesas, Mauritius, Mozambique, Myanmar, Nepal, New Caledonia, Nigeria, Niue, Northern Territory, Ontario, Pakistan, Paraguay, Peru, Philippines, Phoenix Is., Primorye, Puerto Rico, Queensland, Romania, Réunion, Samoa, Senegal, Seychelles, Sierra Leone, Society Is., Solomon Is., Sri Lanka, Sulawesi, Suriname, Tanzania, Tennessee, Thailand, Togo, Trinidad-Tobago, Tubuai Is., Uruguay, USA, Vanuatu, Venezuela, Vermont, Vietnam, Wallis-Futuna Is., Windward Is., Zaïre.

***Ipomoea triloba* L. Sp. Pl.: 161. 1753.**

*Convolvulus trilobus* (L.) Desr. in J.B.A.M.de Lamarck, Encycl. 3:564. 1792. *Quamoclit triloba* (L.) G.Don Gen. Hist. 4:259. 1837. (Fig. 3H)

An annual twining or prostrate herb, glabrous or nodes sparsely pubescent. Petiole 2.5–6 cm, leaf blade broadly ovate to circular, 2.5–7 cm X 2–6 cm, glabrous or sparsely pilose, base cordate. Inflorescences dense umbellate cymes, 1- to several flowered, peduncle 2.5–5.5 cm. Pedicel 5–7 mm, flowers pink or pale purple, funnelform, 1.5–2 cm. Capsule globular, 5–6 mm, bristly pubescent. Seeds dark brown.

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 08 October 2023, Rahman, Sarwar & Ashrafuzzaman 033 (AAHBAU).

**Distribution:** Native to Aruba, Bahamas, Belize, Brazil, Cayman Is., Colombia, Costa Rica, Cuba, Dominican

Republic, El Salvador, Galápagos, Guatemala, Haiti, Honduras, Jamaica, Leeward Is., Mexico, Netherlands Antilles, Panamá, Puerto Rico, Trinidad-Tobago, Turks-Caicos Is., Venezuela, Windward Is.; Introduced into Australia, Borneo, Burkina, Cape Verde, Caroline Is., China, Christmas I., Ecuador, Egypt, Guinea, India, Ivory Coast, Jawa, Kazan-retto, Laos, Lesser Sunda Is., Malaya, Maluku, Marianas, Marshall Is., Mauritius, Myanmar, Nansei-shoto, Nepal, New Guinea, Northern Territory, Palestine, Peru, Philippines, Samoa, Senegal, Sierra Leone, Solomon Is., South China Sea, Sri Lanka, Sulawesi, Sumatera, Taiwan, Thailand, USA, Vietnam, West Himalaya.

***Merremia emarginata* (Burm.f.) Hallier f. Bot. Jahrb. Syst. 16: 552. 1893.**

*Evolvulus emarginatus* Burm.f. Fl. Indica:77. 1768. *Ipomoea emarginata* (Burm.f.) Kuntze Revis. Gen. Pl. 2:443. 1891. (Fig. 3I)

A perennial herb with prostrate stems rooting at the nodes, up to 0.75 m. Leaves reniform to broadly ovate, 0.5–3.5 mm x 0.6–3.5 mm, cordate basally with a broadly rounded sinus and rounded basal lobes, obtuse to broadly rounded or somewhat emarginate apically, coarsely crenate or entire, glabrous or sparsely appressed pilose, petiole 0.2–3.7 cm. Inflorescences solitary or in 2–3-flowered cymose, flowers subsessile, yellow with a paler tube, campanulate, 5–9 mm long. Fruits capsular, subglobose, 5–6 mm long, brownish-black or black. Seeds greyish-brown.

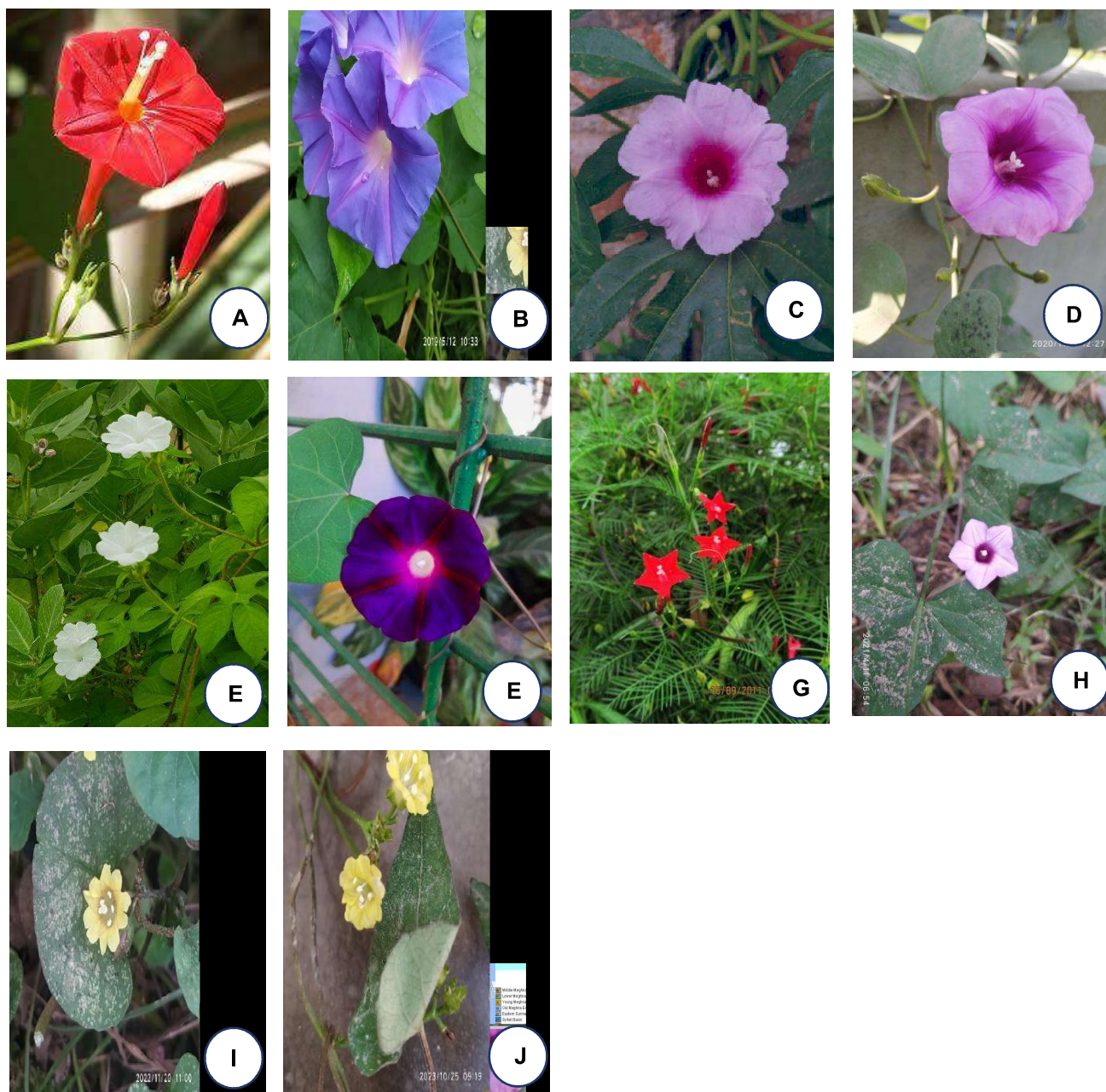
**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 20 November 2022, Rahman, Sarwar & Ashrafuzzaman 004 (AAHBAU).

**Distribution:** Native to Angola, Bangladesh, Borneo, Burkina, Burundi, Cameroon, Chad, China Southeast, Ethiopia, Hainan, India, Jawa, Lesser Sunda Is., Mauritania, Myanmar, Nepal, New Guinea, Philippines, Sri Lanka, Sudan, Sulawesi, Tanzania, Thailand, Uganda, Zaïre; Introduced into Madagascar.

***Merremia hederacea* (Burm.f.) Hallier f. Bot. Jahrb. Syst. 18: 118. 1893.**

*Convolvulus flavus* Willd. Sp. Pl., ed. 4. 1:852. 1798, nom. illeg. *Evolvulus hederaceus* Burm.f. Fl. Indica:77. 1768. (Fig. 3J)

A twining herb, glabrous or sparsely hirsute, rooting at nodes. Leaf 2–4 cm x 1.5–3 cm, cordate-ovate, base cordate or broadly cordate, rarely 3-lobed, petiole 1-2 cm long, glabrous or pubescent. Inflorescences one or few to many-flowered cymes, peduncle 2–4 cm long. Flower yellow, campanulate, c. 1.3 cm across, outside glabrous, inside villous basally. Capsule c. 5 mm long, depressed globose or broadly conical, reticulate wrinkled. Seeds trigonous-globose.



**Fig. 3.** Photographs of different Convolvulaceae species. (A) *Ipomoea hederifolia*, (B) *Ipomoea indica*, (C) *Ipomoea mauritiana*, (D) *Ipomoea pes-caprae*, (E) *Ipomoea pes-tigris*, (F) *Ipomoea purpurea*, (G) *Ipomoea quamoclit*, (H) *Ipomoea triloba*, (I) *Merremia emarginata*, and (J) *Merremia hederacea*

**Specimen examined:** Mymensingh, Bangladesh Agricultural University campus, 25 October 2023, Rahman, Sarwar & Ashrafuzzaman 010 (AAHBAU).

**Distribution:** Native to Australia, Bangladesh, Benin, Borneo, Burkina, Cambodia, Cameroon, Caroline Is., Central African Repu, Chad, China, Christmas I., Congo, East & West Himalaya, Ethiopia, Gabon, Ghana, Guinea, Guinea-Bissau, Hainan, India, Ivory Coast, Japan, Jawa, Kenya,

Laos, Lesser Sunda Is., Liberia, Madagascar, Malaya, Mali, Marianas, Mauritius, Mozambique, Myanmar, Nepal, New Guinea, Niger, Nigeria, Northern Territory, Pakistan, Philippines, Rwanda, Réunion, Senegal, Sierra Leone, Somalia, Sri Lanka, Sudan, Sumatera, Taiwan, Tanzania, Thailand, Togo, Vietnam, Zambia, Zaire, Zimbabwe; Introduced into Colombia, Cuba, Leeward Is., Society Is., Trinidad-Tobago, Windward Is.

## CONCLUSION

The morning glory family Convolvulaceae is represented by a total of 22 taxa belonging to 6 genera at the BAU campus; all taxa are important in both medicine and food crops except blue daze *Evolvulus glomeratus*, the well-known ornamental plant for decades. Green weed management techniques should, therefore, be used to control and utilise these plants (weeds) sustainably without compromising agricultural production and crop yield.

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# Behavioral Responses of *Rhyzopertha dominica* to Different Food Bait Attractants in Paddy Storage Godown

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**Abstract:** This study aimed to evaluate behavioural response of *Rhyzopertha dominica* to crushed groundnut, wheat flour, cracked corn, sorghum flour, rice flour, pearl millet flour, rice bran, rice bran + rice flour, cracked sorghum and control were tested. It showed that wheat flour, cracked sorghum and pearl millet flour were found to be the more attractive. Cracked sorghum exhibited the highest attraction levels, with 26.11% at 5 days after placement (DAP). Wheat flour showed a relatively higher attraction, with 12.79% at 10 DAP. The attraction index is 11.25% at 15 DAP, over the observation period in pearl millet flour. Overall, the results indicate that cracked sorghum (85.07%), wheat flour (57.41%) and pearl millet flour (48.04%) are particularly attractive to *R. dominica*, while other food sources exhibit varying levels of attractiveness. The bait effectiveness was confirmed through testing, revealing varying ratios. Notably, the chamber retained the highest number of *R. dominica* which contained 2:1:1 ratio of wheat flour: sorghum flour: pearl millet flour. The effective baits were also test verified through four-arm olfactometer and found the highest orientation in the arm containing wheat flour and sorghum flour.

**Keywords:** *Rhyzopertha dominica*, Wheat flour, Sorghum flour, Pearl millet flour, Paddy storage godown

Rice is one of the crucial food crops for over half of the global population. The impact of insect-related losses in storage significantly affects food availability. Most commonly the insects, mites, birds, rodents, fungi and moisture are the major problems in storage godowns. Adult beetles, especially stored product insects, exhibit a propensity for seeking shelter in the cracks and crevices of warehouses and storage godowns due to their harborage seeking behavior. Worldwide, chemical control stands out as the most commonly utilized method for managing pests of stored products (Fields and White 2002; Nayak et al., 2020). However, resistance among several species of stored product insects to conventional pesticides has increased over the last few decades (Hagstrum and Phillips 2017, Nayak et al., 2020). Certain populations of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae), have been discovered to exhibit over a 1500-fold increase in resistance to the fumigant phosphine compared to susceptible strains (Opit et al., 2012). *R. dominica* has been documented as a significant pest of stored foods on a global scale, capable of infesting fresh and processed food resources derived from 53 plant species, which can be categorized into 31 families (Edde 2012, Buonocore et al., 2017, Dissanayaka et al., 2020). *R. dominica* feeds on whole kernels of stored cereals, in addition to other commodities such as legumes and tubers. The extent of feeding and damage inflicted by *R. dominica* varies across different types of stored products (Cinco-Moroyoqui et al., 2006, Naseri and Majd-Marani 2022). In principle, this species is regarded as a

particularly significant pest of stored cereals, such as wheat, barley, rice and to a lesser extent in maize (Edde 2012). The process of host selection in insects typically involves chemical stimuli, such as host plant volatiles. Olfactory cues play crucial roles in food-searching by stored product insects, offering vital information to discriminate among different substrates (Hagstrum and Phillips 2017). In this study, the behavioral responses of *R. dominica* to volatiles from different grain commodities, i.e. crushed groundnut, wheat flour, cracked corn, sorghum flour, rice flour, pearl millet flour, rice bran, rice bran + rice flour, cracked sorghum and control (without bait) were assessed in storage godown and four arm olfactometer bioassays. Hence, this study will prove valuable for the advancement of new attractants by enhancing the efficacy of pheromone lures with volatile semiochemicals.

## MATERIAL AND METHODS

Bait traps, filled with attractants, are strategically positioned between the stacked bags in the godown. Designed with a 4mm entrance, they guide insects into the lower polyethylene receptacle. The trap catches were documented at intervals of 5, 10, 15, 20 and 25 days after the placement (DAP) of baits. The quantity of insects captured in the control group, lacking bait material, was juxtaposed with the count of insects captured in the other treatment groups. The trap catches were recorded on 5, 10, 15, 20, 25 days after placement of baits. The number of insects caught in control (without bait material) was compared with the number of insects caught in other treatments (Sathiyaseelan et al.,

2022). The attraction index was calculated by using the formula (Smith et al., 1993).

$$\text{Attraction Index} = \frac{T-C}{N} \times 100$$

T- Number of insects drawn to the treatment.

C- Number of insects drawn to the control

N - Total number of individuals

**Rearing of test insects:** Adults of the lesser grain borer, *R. dominica*, were reared in plastic jars using wheat flour and grains as their diet. Each jar contained 20 to 30 pairs of insects and 250 g of grains, with a piece of kada cloth secured on top with rubber bands. The rearing environment maintained a controlled setting with a 12:12 hour light: dark photoperiod, temperatures ranging between 26 and 28°C, and relative humidity levels maintained at 60 to 65 percent. All experiments were conducted under uniform conditions to ensure consistency in culture maintenance.

**Trapping efficiency of different food bait mixture:** Depending on bait attractiveness, three promising bait sources were selected for further studies. An experimental chamber was arranged with four numbers of container 150 g capacity attached to two litre containers at the centre through PVC pipes with an angle of 45°. The selected food bait materials viz. wheat flour, sorghum flour and pearl millet flour were mixed at the ratios of 1:1:1, 2:1:1, 1:2:1, 1:1:2 (20g each) and filled in the chambers. The test insect *R. dominica* (700) were released in the central arena and the movement of insects towards the food bait mixtures was observed 24 hours after release (HAR).

**Behavioural / Orientation studies:** The test insects, including the lesser grain borer, *R. dominica*, underwent a 24h period of starvation in Petri plates prior to initiating the olfactory bioassay. Fifty adult insects, their sexes undisclosed, were introduced into the central chamber of the olfactometer, where a 7mm aperture was present. The setup was covered with cloth to reduce the insect's attraction to light. At 5, 10, 15, 20, 25 Minutes After Release (MAR), the location of the *R. dominica* was observed (Vijay et al., 2020). Each treatment was replicated 5 times. The reaction of *R. dominica* was evaluated using wheat flour, sorghum flour and pearl millet flour.

**Statistical analysis:** The data on attraction index and behavioural response/orientation of the *R. dominica* beetle were statistically analysed using completely randomized design by one-way ANOVA subjecting the data to arcsine transformation and were separated by using Duncan's multiple range test (DMRT) with IBM SPSS statistics 22.0 software and differences were regarded as significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Different food baits used to study the attraction index for

trapping *R. dominica* revealed that cracked sorghum was the most effective, achieving an attraction index of 26.11% at 5 DAP. Bait traps filled with wheat flour and a mixture of rice bran and rice flour collected 12.79% and 10.94% respectively. The catches for cracked corn and pearl millet flour were 8.86% and 7.73%, while rice bran was the least effective, attracting only 3.89%. By 10 DAP, the attraction index for *R. dominica* was in descending order, with cracked sorghum at 19.41%, followed by wheat flour at 12.79%, and cracked corn at 10.46%, which was comparable to rice bran + rice flour at 10.37%. Crushed groundnut (7.9%), pearl millet flour (7.53%) and sorghum flour (7.17%) also attracted the pests, while rice bran remained the least effective at 3.97%. At 15 DAP, the highest trap catches were recorded for cracked sorghum at 14.66%, followed by wheat flour at 12.76% and pearl millet flour at 11.25%. The lowest catches continued to be observed in rice bran and rice flour. At 20 DAP, cracked sorghum again showed the greatest effectiveness with 12.92%, followed by pearl millet flour (11.15%) and wheat flour (10.82%). Rice bran continued to yield the lowest catches. Finally, at 25 DAP, cracked sorghum recorded the highest trap catches at 11.97%, closely followed by pearl millet flour (10.38%), crushed groundnut (10.35%) and wheat flour (9.52%). Once again, rice bran and rice flour showed the least effectiveness. In total, cracked sorghum achieved an impressive attraction index of 85.07%, followed by wheat flour (57.41%), pearl millet flour (48.04%) and rice bran + rice flour (47.22%), with rice flour having the least attraction at 26.48% (Table 1).

Wheat flour are more attractive than other flours, while millet flour or cracked millet volatiles are more attractive than whole millet kernels Sathiyaseelan et al. (2023). The orientation behavior of the lesser grain borer, *Rhyzopertha dominica*, larger grain borer, *Prostephanus truncatus* and maize weevil, *Sitophilus zeamais* was studied in response to crushed white maize (food odor) combined with color cues (white, yellow, blue, green). While *S. zeamais* responded to both color and odor stimuli, *R. dominica* and *P. truncatus* showed no response (Arnold et al., 2015). The behavioral responses of the lesser grain borer, *Rhyzopertha dominica* and the red flour beetle, *Tribolium castaneum* to sorghum, wheat, and cotton seeds were observed in the field, both near and away from the storage godown. It was found that *T. castaneum* was more attracted to cottonseed than to sorghum or wheat, while *R. dominica* showed a preference for wheat and did not respond to cottonseed (Ahmad et al., 2013).

The *R. dominica* (180 Nos.) 2:1:1 ratio was found to be highly attractive followed by 1:1:1 (165 Nos.) and 1:1:2 (162 Nos.) and both the bait ratio was on par with each other. The



less numbers of *R. dominica* (158 Nos.) retained in 1:2:1 ratio (Table 2). *Rhyzopertha dominica* spent more time inside and entered more areas containing both winter wheat and maize, spending more time in and making more entries into the zones containing these seeds (Ukeh and Umoetok 2007).

**Orientation of stored product insect in four-way olfactometer:** Olfactometer bioassay revealed the significant variations on orientation/behavioural response of *R. dominica* towards wheat flour, sorghum flour, pearl millet flour and control (without food bait) in a four-arm olfactometer. The olfactometer bioassay demonstrated significant variations in the orientation and behavioral responses of *Rhyzopertha dominica* towards wheat flour, sorghum flour, pearl millet flour and a control (without food bait) in a four-arm olfactometer. At 5 minutes after release (MAR), the highest orientation was recorded towards wheat flour at 24%, which was significantly greater than the other flours. This was followed by sorghum flour at 17.3% and pearl millet flour at 16.6%. At 10 MAR, wheat flour continued to attract the most *R. dominica*, with an orientation of 32.1%, followed by sorghum flour at 28.1%. By 15 MAR, the maximum orientation shifted slightly, with wheat flour reaching 36.2%, followed by pearl millet flour at 28.2% and sorghum flour at 22.2%. At 20 MAR, *R. dominica* showed a strong preference for wheat flour at 30.1%, with sorghum

flour following at 26.1%. Finally, at 25 MAR, 38.6% of *R. dominica* settled in wheat flour, succeeded by sorghum flour at 26.6% and pearl millet flour at 18.6% (Table 3).

The four-way olfactometer is utilized to investigate the behavior of *Rhyzopertha dominica* (Bashir et al., 2003). Vijay et al. (2020) reported that the highest orientation of *S. oryzae* was recorded towards sorghum (53.33% and 48.67%) in 20 MAR. According to Trematerra et al. (2000), *O. surinamensis*, *T. castaneum*, and *T. confusum* use grain volatile odours to determine whether stored wheat grain kernels have been damaged mechanically or by insects and these studies are

**Table 2.** Effect of bait source ratio on the arrest of insect movement (wheat flour: sorghum flour: pearl millet flour)

Bait ratio	Number of percent recaptured*
1:1:1	165±3.9863 <sup>b</sup>
2:1:1	180±5.5113 <sup>a</sup>
1:2:1	158±3.2251 <sup>c</sup>
1:1:2	162±1.7636 <sup>b</sup>
Total	665
Not responded	35

\* Based on 700 insects  
Means followed by the same letter (s) in a column are not significantly different by DMRT (P=0.05)

**Table 1.** Comparative response of *Rhyzopertha dominica* to various food attractants

Attractants	Relative attraction index (%)					Total attraction (%)
	5 DAP *	10 DAP	15 DAP	20 DAP	25 DAP	
Crushed groundnut	5.34±0.053 <sup>g</sup>	7.9±0.116 <sup>d</sup>	9.29±0.016 <sup>e</sup>	9.66±0.108 <sup>d</sup>	10.35±0.256 <sup>b</sup>	42.54
Wheat flour	11.57±0.079 <sup>b</sup>	12.79±0.305 <sup>b</sup>	12.76±0.122 <sup>b</sup>	10.82±0.317 <sup>c</sup>	9.52±0.291 <sup>c</sup>	57.41
Cracked corn	8.86±0.006 <sup>d</sup>	10.46±0.142 <sup>e</sup>	8.84±0.180 <sup>f</sup>	8.73±0.042 <sup>e</sup>	8.46±0.248 <sup>e</sup>	45.35
Sorghum flour	6.29±0.120 <sup>f</sup>	7.17±0.117 <sup>f</sup>	9.41±0.275 <sup>e</sup>	8.77±0.149 <sup>e</sup>	8.79±0.084 <sup>d</sup>	40.43
Rice flour	4.54±0.148 <sup>b</sup>	6.16±0.130 <sup>g</sup>	5.27±0.104 <sup>g</sup>	5.43±0.129 <sup>b</sup>	5.08±0.076 <sup>b</sup>	26.48
Pearl millet flour	7.73±0.110 <sup>e</sup>	7.53±0.097 <sup>e</sup>	11.25±0.299 <sup>c</sup>	11.15±0.243 <sup>b</sup>	10.38±0.099 <sup>b</sup>	48.04
Rice bran	3.89±0.119 <sup>g</sup>	3.97±0.005 <sup>h</sup>	5.38±0.143 <sup>g</sup>	6.72±0.146 <sup>g</sup>	7.96±0.087 <sup>f</sup>	27.92
Rice bran + Rice flour	10.94±0.089 <sup>c</sup>	10.37±0.000 <sup>c</sup>	9.8±0.267 <sup>d</sup>	8.41±0.195 <sup>f</sup>	7.7±0.042 <sup>g</sup>	47.22
Cracked sorghum	26.11±0.764 <sup>a</sup>	19.41±0.647 <sup>a</sup>	14.66±0.020 <sup>a</sup>	12.92±0.273 <sup>a</sup>	11.97±0.277 <sup>a</sup>	85.07
Control	0.00	0.00	0.00	0.00	0.00	0.00

\*DAP – Days after placement; Means followed by the same letter (s) in a column are not significantly different by DMRT (P=0.05)

**Table 3.** Behavioural/orientation response of *Rhyzopertha dominica* to various food attractants

Food attractants	<i>Rhyzopertha dominica</i> settled (%)				
	5 MAR*	10 MAR	15 MAR	20 MAR	25 MAR
Wheat flour	24.6 ±0.347 <sup>a</sup>	32.1±0.226 <sup>a</sup>	36.2±1.002 <sup>a</sup>	30.1±0.656 <sup>a</sup>	38.6±0.318 <sup>a</sup>
Sorghum flour	17.3 ±0.565 <sup>b</sup>	28.1±0.267 <sup>b</sup>	22.2±0.513 <sup>c</sup>	26.1±0.603 <sup>b</sup>	26.6±0.325 <sup>b</sup>
Pearl millet flour	16.6±0.169 <sup>c</sup>	14.1±0.182 <sup>c</sup>	28.2±0.287 <sup>b</sup>	20.1±0.136 <sup>c</sup>	18.6±0.101 <sup>c</sup>
Control (without food)	6.6±0.004 <sup>d</sup>	9.5±0.181 <sup>d</sup>	4.9±0.106 <sup>d</sup>	7.4±0.211 <sup>d</sup>	8.0±0.152 <sup>d</sup>
Unsettled	48.0	26.8	19.5	26.8	21.3

\*MAR- Minutes after release

corroborative to our findings. Sathiyaseelan et al. (2024), who demonstrated odour-based host searching behaviour of *R. dominica*, *T. castaneum* where all of them were attracted by the trap odour emanated from wheat flour. The highest orientation recorded was 28.5% toward wheat flour, which was significantly greater than that of other flours. This was followed by sorghum flour at 21.16% and pearl millet flour at 17.5% (Sathiyaseelan et al. 2024).

### CONCLUSIONS

This study highlights the significant behavioral responses of the lesser grain borer, *Rhyzopertha dominica*, to various food bait attractants in storage environments. Cracked sorghum and wheat flour emerged as the most attractive substrates, demonstrating the importance of specific grain volatiles in guiding the orientation of *R. dominica*. The findings indicate that the attraction indices varied over time, with distinct preferences observed for different bait materials. This knowledge can be instrumental in developing effective monitoring and control strategies for managing *R. dominica* populations in storage facilities. By incorporating attractive volatile semiochemicals into pheromone lures, it is possible to enhance the efficacy of pest management practices, ultimately contributing to improved food security and reduced losses in stored products. The insights gained from this research could inform future studies on sustainable pest management approaches and promote the development of more effective and environment friendly attractants for stored product insects.

### AUTHOR CONTRIBUTION

The study was conceptualised, designed and carried out the experiments by M. Sathiyaseelan. K. Balaji assisted with the data collection and data analysis. The article was read and approved by all the authors.

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# Principal Component Analysis of Morphological and Physiological Traits in Mustard (*Brassica juncea*) in Semi-arid Condition of Rajasthan

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**Abstract:** Mustard (*Brassica spp.*) is a vital oilseed crop with significant variability in key morphological, physiological, and yield-related traits, offering substantial scope for genetic improvement. This study employed descriptive statistics and principal component analysis (PCA) to analyze different traits. The first two components accounted for 59.8% of the total variability, with PC1 (51.5%) emphasizing growth traits like days to maturity and PC2 (8.2%) highlighting quality traits such as oil content and 1000-seed weight. Genotypes such as IC 122449 and its hybrids showed promise for early maturity and biomass accumulation, while PM 28 × EC 766136 excelled in yield and quality traits. Correlation analysis revealed key relationships, including a negative correlation between days to flowering and seed yield (-0.396) and positive correlations for drought-resilience traits like relative water content (0.52) and proline content (0.507) with yield. These findings underscore the importance of integrating multi-trait analysis for breeding stress-tolerant, high-yielding, and quality-rich mustard varieties.

**Keywords:** Mustard, *Brassica juncea*, Traits, Principal component analysis, Semi-arid condition

Mustard (*Brassica spp.*) is a vital oilseed crop in India, contributing significantly to edible oil production and bolstering the agricultural economy (Rathore et al., 2020). Major growing regions include Rajasthan, Uttar Pradesh, and Haryana, where it plays an essential role in sustaining rural livelihoods (Singh et al., 2022). Internationally, mustard is valued for its resilience to varied climatic conditions and its high-quality oil and meal products (Meena et al., 2021). The crop exhibits substantial variability in morphological, physiological, and yield-related characteristics, presenting excellent potential for genetic enhancement (Yadav et al., 2020). Breeding initiatives are focused on developing varieties with higher yields, improved stress tolerance, and superior quality to meet the increasing demand for edible oil (Kumar and Singh, 2020). Harnessing the genetic diversity within mustard genotypes is key to addressing challenges such as climate change, pest infestations, and diseases (Verma et al., 2021). Principal Component Analysis (PCA) is a robust statistical tool commonly used to analyze complex datasets in plant breeding research (Patel and Mehta, 2020). It effectively reduces the data's dimensionality while preserving critical information, which aids in identifying the primary traits contributing to variability (Chaudhary et al., 2019). In mustard breeding studies, PCA has been employed to assess traits such as days to flowering, plant height, siliquae per plant, seed yield, and oil content (Rai et al., 2021). These traits are pivotal for breeding programs focused on increasing yield, enhancing stress resistance, and

improving oil quality (Gupta et al., 2020). This research underscores the importance of PCA in understanding the trait architecture of mustard genotypes. By identifying key traits that influence plant performance, PCA provides valuable insights that inform breeding programs aimed at improving yield, quality, and adaptability to diverse agro-climatic conditions (Sharma and Singh 2021). The objective of present study is to analyze the diversity among mustard genotypes. This will help identify major traits for enhancing yield, quality, and adaptability.

## MATERIAL AND METHODS

The experiment was conducted during the *Rabi* season of 2022-23, SKN College of Agriculture, Jobner, Jaipur, Rajasthan. It aimed to assess the variability among Indian mustard genotypes through principal component analysis (PCA). Fifty-five Indian mustard genotypes were evaluated in a randomized complete block design with three replications. The row-to-row and plant-to-plant spacing were maintained at 45 cm and 20 cm, respectively. Standard cultural practices were followed to ensure healthy crop growth. Data were recorded for thirteen morphological and physiological traits (Table 1). Observations for days to 50% flowering, days to maturity, proline content, oil content, and 1000-seed weight were collected on a whole-plot basis. Data for other traits were recorded from ten randomly selected competitive plants in each plot across all replications. The data were analyzed using PCA to assess the contribution of individual traits to

total variability. PCA was performed on a correlation matrix to standardize the variables for comparability. Eigenvalues and the percentage of variance explained by each principal component were calculated. Major traits contributing to genetic variability were identified through PCA. Traits with high factor loadings in the principal components were considered major contributors to variability.

## RESULTS AND DISCUSSION

**Descriptive statistics for PCA analysis of mustard:** The descriptive statistics of various morphological and physiological traits in mustard revealed significant variability (Table 1), which can be used for genetic improvement. Traits like days to 50% flowering (47.44) and days to maturity (120.98) showed moderate variability, providing opportunities to select genotypes suitable for different environments (Singh et al., 2020). Plant height (143.75 cm) and siliquae per plant (191.89) exhibited higher variance, indicating substantial genetic diversity that can be exploited in breeding programs to improve yield (Kumar et al., 2019). In contrast, branches per plant (3.84) and seeds per siliqua (12.25) displayed lower variability, but their stability makes them valuable in selecting yield components (Sharma et al., 2018). Physiological traits like relative water content (81.39%) and membrane stability index (68.93%) demonstrate the potential for selecting drought-tolerant genotypes (Choudhary et al., 2022), while proline content (7.78%) highlights the significance of stress indicators in breeding for abiotic stress tolerance (Bhardwaj et al., 2020). Oil content (36.36%) showed moderate variability, suggesting opportunities to improve oil yield through breeding (Sharma et al., 2018). Overall, the observed

variability in seed yield per plant (45.17 g) indicates potential for selecting high-yielding genotypes in mustard breeding programs aimed at enhancing productivity.

**Correlation analysis of morphological and physiological traits:** The correlation matrix heatmap of mustard traits reveals important relationships that could guide breeding efforts for yield improvement (Fig. 1). Days to 50% flowering (DF) showed a negative correlation with seed yield (Sy) (-0.396), suggesting early flowering may enhance yield by avoiding terminal drought stress, a trend also observed in mustard studies (Kumar et al., 2020). Plant height had a negative correlation with seed yield (-0.337), indicating that excessive vegetative growth may reduce yield potential (Singh et al. 2018). Branches per plant (Br) and siliqua per plant exhibited positive correlations with yield (0.246 and 0.458, respectively), reinforcing their critical role in yield determination, similar to findings by Chauhan et al. (2019). Physiological traits such as relative water content (RWC) and membrane stability index (MSI) were positively correlated with seed yield (0.52 and 0.301), highlighting the importance of drought tolerance for maintaining yield under stress conditions (Sharma et al., 2021). Proline content, which is associated with stress tolerance, also showed a moderate positive correlation with yield (0.507), indicating its role in improving performance under stress (Ashraf and Foolad 2007). Oil content had a weak correlation with seed yield suggesting that oil content can be improved independently without significantly affecting yield, as observed by Yadav et al. (2022). These interrelationships underscore the complexity of breeding for yield improvement and highlight the need to balance agronomic and physiological traits to achieve optimal outcomes.

**Table 1.** Descriptive statistics for agro-morphological and physiological traits in plants

Character	Mean	Variance	Standard deviation	Standard error
Days to 50% flowering	47.44	6.66	2.58	0.35
Days to maturity	120.98	27.18	5.21	0.70
Plant height (cm)	143.75	200.25	14.15	1.91
Branches per plant	3.84	0.16	0.40	0.05
Siliquae per plant	191.89	1029.22	32.08	4.33
Siliquae length (cm)	5.40	0.11	0.33	0.05
Seeds per Siliqua	12.25	0.65	0.80	0.11
1000-seed weight (g)	3.67	0.07	0.27	0.04
Relative Water Content (%)	81.39	10.86	3.30	0.44
Membrane stability index (%)	68.93	11.91	3.45	0.47
Proline content (%)	7.78	1.06	1.03	0.14
Oil content (%)	36.36	3.26	1.81	0.24
Seed yield per plant (g)	45.17	10.99	3.32	0.45

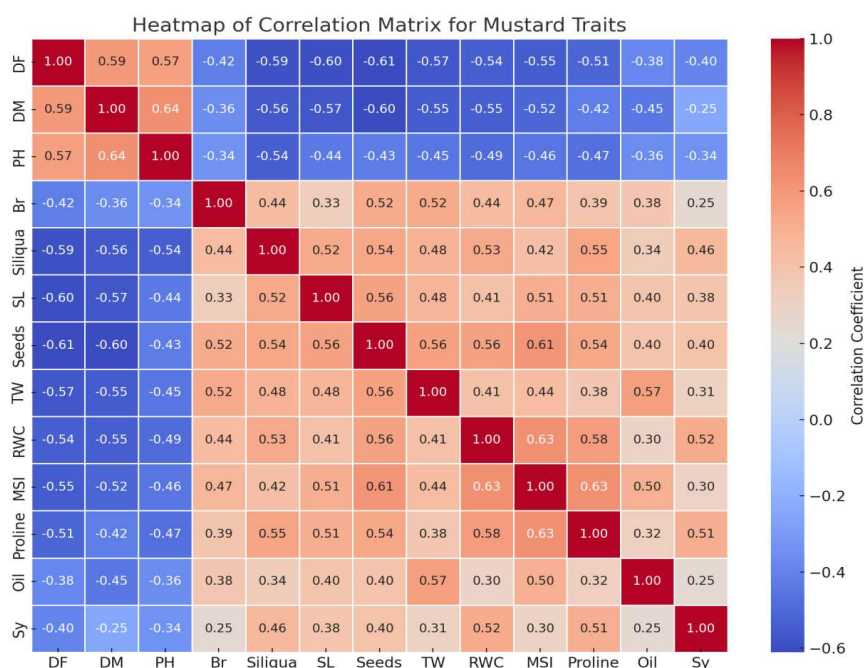
**Principal component analysis of mustard traits variability and breeding implications:** The principal component analysis (PCA) conducted on mustard provided valuable insights into the underlying structure of the data with the first principal component demonstrating an eigenvalue of 6.824 and accounting for 51.5% of the total variability (Table 2, Fig. 2). This significant proportion highlights PC1's role in capturing key traits such as seed yield, siliquae per plant, and oil content, which are critical for breeding programs focused on yield and quality improvement (Kumar et al., 2019, Singh

et al., 2020). PCA's ability to reduce complex datasets into a smaller number of informative components enables the identification of traits that drive variability, guiding targeted breeding efforts. The second principal component exhibited an eigenvalue of 1.091 and contributed an additional 8.2% to the total variability which resulted in a cumulative explained variability of 59.8%. The substantial contribution of these two principal components underscores the importance of specific traits that are likely related to growth or yield or environmental adaptation in explaining the variability among mustard genotypes (Jain et al., 2020). The subsequent components (PC3 to PC12) displayed diminishing returns regarding explained variability, with PC3 accounting for only 6.5% and PC4 for 5.5% of the total variability. The cumulative proportion of variability explained reached 98.5% by the twelfth principal component. This trend suggests that the initial components provide essential information whereas the later components may capture less significant variations (Kumar et al., 2019).

The principal component analysis (PCA) conducted on mustard data provided significant insights into the interrelationships among thirteen characters of the crop, shedding light on the phenotypic traits that contribute most to the overall variability within the dataset. The first principal component (PC1) accounted for a substantial portion of the variance (Table 3), with high positive loadings for days to 50% flowering (0.796) and days to maturity (0.768), indicating their crucial role in the growth and developmental dynamics of mustard. These results highlight the importance of early flowering and maturity traits, which are critical for optimizing

**Table 2.** Eigen values and variability explained by principal components in mustard traits

Principal components	Eigenvalue	Proportion of variability explained	Cumulative proportion
PC1	6.824	0.515	0.515
PC2	1.091	0.082	0.598
PC3	0.864	0.065	0.663
PC4	0.727	0.055	0.718
PC5	0.69	0.052	0.77
PC6	0.628	0.047	0.817
PC7	0.496	0.037	0.855
PC8	0.418	0.032	0.886
PC9	0.381	0.029	0.915
PC10	0.362	0.027	0.943
PC11	0.329	0.025	0.967
PC12	0.239	0.018	0.985
PC13	0.193	0.015	1



**Fig. 1.** Correlation matrix analysis of mustard traits for yield improvement and stress tolerance

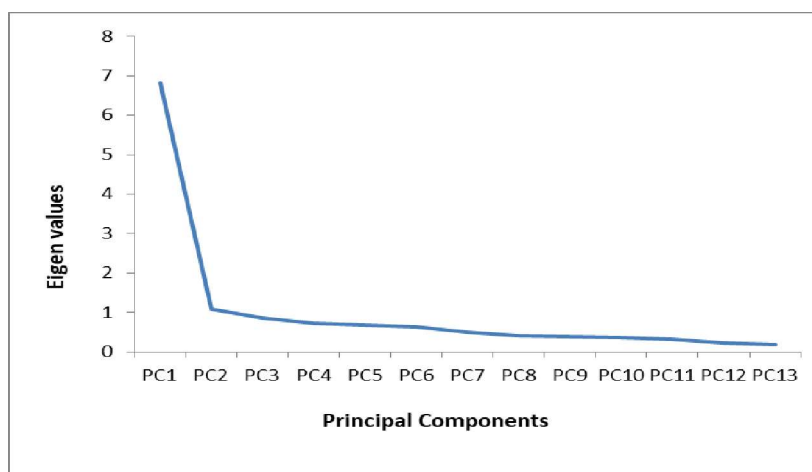
planting and harvesting schedules, particularly in diverse climatic conditions (Bhaduri et al., 2021). The PCA results elucidate the complex relationships among various traits in mustard, emphasizing the significance of specific phenotypic characteristics in determining overall plant performance. The strong positive loadings of flowering and maturity traits on

**Table 3.** Factor loadings of principal components for 13 characters in mustard

Characters	PC1	PC2
Days to 50% flowering	0.796	-0.009
Days to maturity	0.768	-0.203
Plant height (cm)	0.704	0.006
Branches per plant	-0.623	0.232
Siliqueae per plant	-0.751	-0.162
Siliqueae length (cm)	-0.724	0.007
Seeds per Siliqua	-0.795	0.04
1000-seed weight (g)	-0.721	0.408
Relative Water Content (%)	-0.753	-0.312
Membrane stability index (%)	-0.761	0.02
Proline content (%)	-0.734	-0.361
Oil content (%)	-0.593	0.487
Seed yield per plant (g)	-0.561	-0.563

PC1 underscore their importance in mustard's growth cycle, confirming earlier research that indicates that early maturity and flowering time are essential for improving crop resilience and yield potential (Kumar et al., 2019). In contrast, several traits exhibited strong negative loadings on PC1, including seeds per siliqua (-0.795), siliquae per plant (-0.751), and relative water content (-0.753). This negative correlation suggests potential interactions between yield-related traits and other growth parameters which are essential for breeding programs aimed at maximizing mustard yield (Sharma et al., 2021).

The second principal component (PC2) revealed a different trait profile with 1000-seed weight (0.408) and oil content (0.487) showing positive loadings. This suggests that these traits significantly influence another dimension of variability related to quality characteristics, which are increasingly important for market preferences. The negative loading of seed yield per plant (-0.563) on PC2 indicates an inverse relationship between yield and oil content, posing challenges for breeders striving to enhance both yield and quality traits (Ghosh et al., 2018). The weak loadings for



**Fig. 2.** Scree plot showing eigenvalues of principal components in mustard traits

**Table 4.** Principal component scores (PC1 and PC2) for selected genotypes

Genotypes	PC1 scores	Genotypes	PC2 scores
IC 122449	5.574	PM 28 X EC 766136	2.499
IC 122449 X RAJAT	5.203	BPR 349-9 X RAJAT	1.764
IC 122449 X EC 766136	4.483	BPR 543-2 X LAXMI	1.661
IC 122449 X LAXMI	4.4	PM 25 X IC 122449	1.643
PM 25 X IC 122449	3.396	PM 25 X PM 28	1.534
RAJAT	3.026	NRCHB 101 X RAJAT	1.423
BPR 543-2 X IC 122449	2.377	PM 28 X IC 122449	1.33
EC 766136	2.323	BPR 543-2 X PM 25	1.29
LAXMI	2.314	RAJAT	1.229
PM 28	2.023	BPR 543-2 X NRCHB 101	1.225

branches per plant (0.232) and siliquae length (0.007) on both principal components suggest that these traits may have a lesser impact on the primary axes of variability in this dataset.

PCA revealed significant variation among genotypes for studied traits (Table 4). The highest PC1 scores were observed in IC 122449 (5.574) followed by IC 122449 × RAJAT, and IC 122449 × EC 766136. These genotypes excel in early growth traits like plant height, flowering, and maturity. High PC2 scores were seen in PM 28 × EC 766136 (2.499) followed by BPR 349-9 × RAJAT and BPR 543-2 × LAXMI. These genotypes highlight traits such as seed yield, oil content, and stress tolerance. The clustering of IC 122449 and its derivatives on PC1 shows potential for early growth and biomass traits. PM 28 × EC 766136 and PM 25 × IC 122449, with strong PC2 scores, are promising for yield and quality. Singh *et al.* (2020) emphasized the importance of multi-trait integration in selection. This study highlights genetic diversity and its utility in breeding programs. Genotypes like PM 25 × IC 122449 balance traits across PCs, making them suitable for multi-trait hybrids. Results align with Kumar *et al.* (2018) and Verma and Patel (2021). Future breeding should focus on these genotypes to enhance genetic gain and adaptability.

### CONCLUSION

The significant genetic variability in mustard traits such as plant height, siliquae per plant, and oil content offers opportunities for targeted breeding programs. PCA identified key traits influencing variability, with early flowering and maturity playing crucial roles in adaptability. Correlation analysis highlighted important relationships, including the positive impact of drought tolerance traits on yield and the trade-off between oil content and seed yield. Promising genotypes like IC 122449 and PM 28 × EC 766136 show potential for improving growth, yield, and stress resilience. These insights guide breeders in developing mustard varieties that balance yield, quality, and adaptability.

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# Evapotranspiration and Responses to Irrigation of Headed Broccoli under Indian Hot and Sub-Humid Climatic Condition

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**Abstract:** Crops grown under irrigation rather than the monsoon season in India is not an easy farming practice, because of water scarcity under changing climate and market limitations. The present experiment was designed and executed to measure the water use (evapotranspiration) of heading broccoli under furrow irrigation and different water saving techniques. In general, consumptive water use reached a maximum of 118 mm and a minimum of 55 mm for the period of 70 days after transplanting. Single crop coefficient ( $K_c$ ) values were recorded higher than calculated FAO- $K_c$  values during initial, mid and end stages. The response of given irrigation water through flood irrigation method (four treatments, from 25 to 100% of  $ET_c$ ) to the yield of broccoli, formed a production function that gave the highest yields (near 15 t ha<sup>-1</sup>) at an irrigation level of 118 mm, and a 12.61 kg m<sup>-3</sup> water use efficiency. Broccoli cultivation during the winter season is an economically feasible alternative for diversification of crops around the year at a time where irrigation water demand for other crops is maximum.

**Keywords:** Broccoli,  $SET_{\text{watbal}}$ ,  $SET_{\text{FAO}}$ , WUE,  $K_c$

Across the globe, increasing population, urbanization, modernization in developing countries has created a significant risk on water quality. Globally, 44 countries are projected to either extremely high or high-water stress level (ratio of water withdrawals to water supply) in 2040 and India is one of them, having a high water stress level country (Rana and Bhardwaj 2021, Armstrong 2022). Due to water related losses in agriculture, health, income and prosperity, some regions of the world may see their growth rates decline by 6 % of GDP by 2050 (WB 2023). Increasing urbanization, civilization and industrialization of India, decreases drastically share of fresh water for agriculture, and it will be declined up to 78 % in the year 2050 as against increasing demand 40 % (Jaybhaye et al., 2023). Use of ground water in agriculture causes arsenic contamination in India, and which also deteriorates the environment. Under such situations, more food, fruits and fresh vegetables production need to increase with less available water resources. Considering these actualities, avoidance of the peak summer  $ET_o$  demand, could mitigate aquifer over exploitation and make irrigation more sustainable by reducing seasonal irrigation to growing various crops.

Depletion in evapotranspiration rate by using the ET reducing techniques may increase water productivity, but water productivity can also be improved by increasing its yield or the gross income (Jaybhaye et al., 2023). Areas of water scares, a shifting from traditional field crops into higher valued horticultural crops has been observed (Feres et al., 2003). Therefore, an accurate quantification of crop

evapotranspiration is supportive for proper planning and management of irrigation (Meshram et al., 2018). Reduction of water losses in agricultural crop production is highly valuable to lessen impact of water scarcity and changing climatic condition. Among different crops, horticultural crops are considered as the best, suitable alternative to the urgently needed balanced diversification along with the staple food of Indian agriculture. Similarly, introducing a horticultural crop like broccoli with their high cost-effectively (and risk) in an area mainly devoted to annual field crops could rejuvenate a sector vulnerable to decrease in the subsidies from the Common Agricultural Policy of the West Bengal. Therefore, it's required accurate calculation of crop water requirements, usually used the standard FAO approach (Allen et al., 1998a) that uses  $ET_o$  and a crop coefficient ( $K_c$ ).

Information on  $K_c$  is widely available for many crops and it was originally obtained by measuring crop ET, usually with lysimeters, and then relating it to  $ET_o$ ). The progression of  $K_c$  over time during the crop growing season is represented by  $K_c$  curve. Lopez-Urrea et al. (2009) described that the measured  $K_c$  values at any rate, do not best fit the linear or even the curvilinear model, as there are significant fluctuations above and below the fitted lines. From the above, it appears advisable to carry out regional or local alterations of broccoli  $K_c$  for improvement in the estimates of ET precisely. Therefore, there is the need for the assessment of  $K_c$  value of any crop for a particular region. Likewise, it would be important to clarify the response of this crop to different



irrigation regimes to assess the ideal level of water application to fall plantings. Therefore, we conducted an experiment to (a) determine the Kc value of broccoli crop and (b) to evaluate the response of broccoli to deficit irrigation.

### MATERIAL AND METHODS

The research experiment was carried out during 2016-17 and 2017-18 (during the period of November to January) at Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, (India) to find out response of irrigation regimes (IR) and water saving techniques (WST's). The experimental site climate is hot and sub-humid, continental with 1600 mm of annual average rainfall and 85 % of it is received during the monsoon period i. e. from 18-24 June (25<sup>th</sup> SMW) to 24-30 September (39<sup>th</sup> SMW). January is the coldest month with a mean temperature value ranging from 15.5°C to 21.3°C. The atmospheric mean temperature begins to rise towards the beginning of February and reaches its mean maximum (27.6 to 31.1 °C) during May. Higher mean relative humidity (97%) experienced during the 50<sup>th</sup> to 3<sup>rd</sup> standard meteorological week (SMW) and is at the lowest level (39%) in the 10<sup>th</sup> SMW. Normal pan evaporation value reaches its maximum level 3.4 mm/day during the 10<sup>th</sup> SMW and it remains at its lowest level (1.0 mm/day) during the 51<sup>st</sup> SMW. While, the 47<sup>th</sup> and 48<sup>th</sup> SMW were the driest period with no rainfall; whereas, the maximum rainfall received during the 46<sup>th</sup> SMW. The experimental plot soil was classified as a sandy loam hyperthermic Aeric Haplaquept (Jaybhaye and Mukharjee 2020). Average depth of the soil at the experimental site was >100 cm. The Texture was sandy-clay-silt, in general with 56% of sand, 21% of silt and 23% of clay, and the basic pH was 5.5 to 6.5. The soil was rich in organic matter as well as in nitrogen. Experimental sites geo-coordinates and experimental details (viz., irrigation treatments and water saving techniques as sub treatments details, statistical design, agronomical practices, harvesting etc.) are given thoroughly by Jaybhaye et al. (2023).

Gravimetric soil water content was measured from 0-150, 150-300, 300-450 and 450-600 mm depths weekly in between period of sowing and harvest; as well as before and after each irrigation and after notable ( $\geq 20$  mm) rainfall. During the whole cropping period (sowing to harvest) seasonal evapotranspiration (SET) from the crop field was calculated by using the field water balance equation.

$$ET = P + I + C - D \pm \Delta SWS \quad (1)$$

Where, P- precipitation (mm), I- total irrigation water applied (mm), C- capillary contribution (mm), D- vertical drainage (mm) and  $\Delta SWS$  - depletion in soil water storage (mm). It was considered, the capillary contribution and deep drainage contribute negligible amount to the total seasonal

evapotranspiration value for this region (Mukherjee et al 2010). Hence, not considered both C and D in the present study. The resulting data was computed for measurement of broccoli ET under the regional climatic condition. Cumulative GDD (°C day) was computed by using the 3.0°C  $T_{base}$  and the equation which was earlier used by Diputado et al (1989).

$$GDD = \sum_{x=1}^n [(TD_{max} + TD_{min})/2] - T_{base} \quad (2)$$

Where,  $T_{base}$  is the base temperature of broccoli;  $TD_{max}$  is the maximum temperature of the day and  $TD_{min}$  is the minimum temperature of the day.

Statistical analysis: Entire collected data was analyzed by using SAS (ver. 9.3, SAS, Inc., Cary, NC) computer package program. The statistical measurements of coefficient of determination ( $R^2$ ) of the equations was determined to show the proportion of the variation in the dependent variable that is predictable from the independent variable, and descriptive analysis was done with the new Microsoft Excel (Windows v. 10.0) to indicate the degree of association between two variables (i.e. dependent and independent variable).

### RESULTS AND DISCUSSION

**Evapotranspiration:** The seasonal ET records of broccoli as well as the water contribution from irrigation and rainfall is depicted in Figure 1. The within SET values which were obtained by  $SET_{watbal}$  and  $SET_{FAO}$  (Fig. 2), shows a very good relationship for the seasonal comparison and the similar results were reported by Lopez-Urrea et al. (2009). The measured mean  $ET_c$  for the crop growing season were between 1.4 and 2.0 mm per day under  $I_{0.25}$  and  $I_{1.00}$ , irrigation regimes respectively. The seasonal mean calculated values was 1.6, 1.3, 0.9 and 0.7 mm per day under  $I_{1.00}$ ,  $I_{0.75}$ ,  $I_{0.50}$  and  $I_{0.25}$  irrigation regimes respectively and the slope of the regression line was very close to unity (Fig. 2).  $SET_{watbal}$  shows an overestimate compared to the  $SET_{FAO}$  (22, 30, 44 and 46 percent under  $I_{1.00}$ ,  $I_{0.75}$ ,  $I_{0.50}$  and  $I_{0.25}$  irrigation regimes respectively). The irrigation regime wise individual value of  $SET_{watbal}$  showed significant increment seasonal it's value shows a very good agreement and differ results from those reported by Lopez-Urrea et al (2009). Figure 2 depicts the linear relationship between measured values by  $SET_{watbal}$  and obtained values through  $SET_{FAO}$  and similar results were shown by Lopez-Urrea et al (2009). The regression equation shows that, about 25 percent variation in  $SET_{FAO}$  can be explained by  $SET_{watbal}$  value. It may be due to the differing climatic conditions at a specific location (Kalyani) than the referred climate for calculation of  $SET_{FAO}$ . Lopez-Urrea et al (2009) also emphasized on conduct regional studies for improvement in the precision estimation of ET in broccoli

crop.  $SET_c$  by water balance -  $SET_c$  by FAO relationship obtained for broccoli crop is -

$$SET_{FAO} = 1.97 SET_{watbal} - 32.749; R^2=0.75 \quad (3)$$

**Single crop coefficient curves:** Besides, soil water redistribution processes were unaffected when the crop was grown under control condition. For this reason, out of twenty combinations AET under  $I_{1.00}$ - $M_c$  combination was used to compute the  $K_c$  values of broccoli. During 1<sup>st</sup> and 2<sup>nd</sup> year, drying cycles were recorded 5 and 4, respectively. Thus, we got a total of nine sets of  $K_c$  value, which was computed based on our experiments.  $K_c$  values of the broccoli crop at a 7-day interval averaged for three important crop growth stages viz., initial (Rosette development, RSD), mid (Heading, HD) and end (Maturity, HT) were 0.94, 1.46 and 1.63 at RSD, HD and HT respectively. In comparison to the FAO- $K_c$  values were higher by 26, 28 and 48% during initial, mid and end stages, respectively. The  $K_c$  curves fit to the  $K_c$  values computed by using measured  $SET_{watbal}$  and which does not follow the conventional shape of the crop coefficient curve recommended (FAO- $K_c$ ) for broccoli (Fig. 3). Subsequently, the obtained  $K_c$  value was observed highest at commercial harvest recommended FAO- $K_c$ , with a slightly declining rate, differing in  $K_c$  values recommended by Allen et al. (1998b). During the present field experiment, broccoli heads were harvested at terminal head maturity stage, where the leaf area index and foliage growth remain at the highest level. It may be the probable reason for having higher  $K_c$  value at later crop growth stage (harvesting stage) and these results are similar to the results of Lopez-Urrea et al (2009). The extrapolation of the results of present investigation may be useful to other areas and the  $K_c$  values computed from the water balance method were fitted to the evaluating of GDD values (Fig. 4), which tracks the crop development pattern. The relationship obtained between GDD and water balance  $K_c$  for a broccoli crop is;

$$K_c = -7E^{-6} GDD^2 - 0.009GDD + 3.644; R^2 = 1 \quad (4)$$

By reducing crop canopy temperature, reduction in GDD and early maturity period (1-2 week) are possible, and vice-versa to escape crop from weather abortion events (i.e. unseasonal dramatic change in weather parameters), which helps for getting more yield and also for getting profitable price in market to producers by adjusting harvesting period according to market demand as well as non-congenial probable weather condition. The reduction of GDD values by 30-50% under supply of ample of water in broccoli crop by adjustment of irrigation schedule.

**Net head yield response to applied water and water use efficiency of broccoli:** Regardless of irrigation regimes and water saving techniques, 15.03 t/ha NHY was obtained in 2016-17, which was 29 % lower in 2017-18. During the

second experimental year, the overall temperature was 0.5 to 2.5°C lower compared to the first year, which caused less number of leaf (Tan et al., 2000). During the second year, unexpected rainfall (37.0 mm) occurred just after

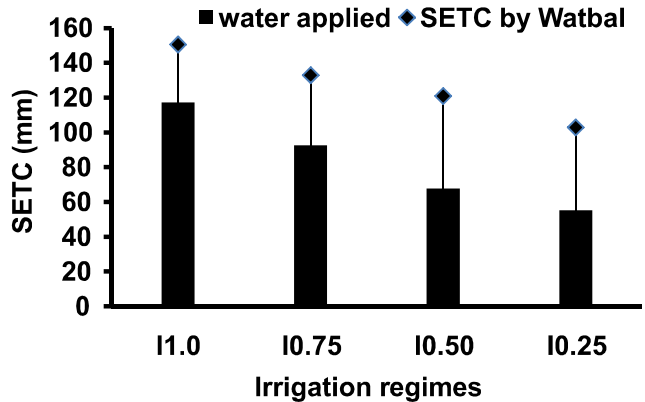


Fig. 1. Seasonal ET values measured by water balance method, water applied (irrigation + rainfall) in mm

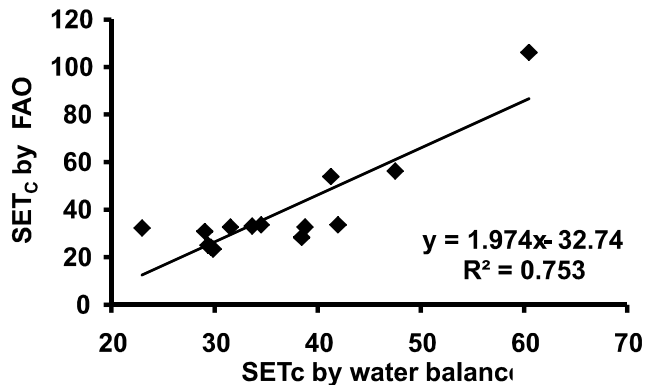


Fig. 2. Simple regression analysis of calculated SETc (mm) by FAO over measured and water depletion method

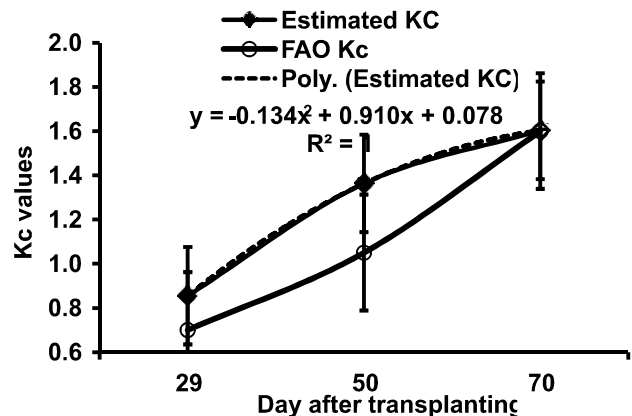


Fig. 3. Crop coefficient curves: (a) recommended in Allen et al (1998a; circles), (b) recommended for fall plantings based on the data measured in the water balance method (squares). Bars indicate the standard error

transplanting, which caused 20 % seedling mortality. Thus, re-transplanting was done in 2017-18. Re-transplanted crops took more days to establish and also the productivity of those plants was not at par with the first transplanted crop. This might be another reason for lower net head yield during the second year of the experimentation. In general, during the second year of experimentation, the length of seasonal duration and prevailed weather parameters (prominently temperature) variation was observed more than those that are common in the first year of experimentation, and might be the major reason to record lower NHY during the second year and the similar reason was quoted by Lopez-Urrea et al (2009) for broccoli.

Irrespective of water saving measures, variation in two years average of NHY was found statistically significant among the irrigation regimes (Table 1). The maximum NHY (15.17 t/ha) observed under  $I_{1.0}$ , which was at par with  $I_{0.75}$  (14.30 t/ha). The NHY of broccoli decreased significantly by 19 and 35 % with a decrease in status of soil water stress respectively under  $I_{0.50}$  and  $I_{0.25}$  treatments. The NHY was recorded at par within the  $I_{1.0}$  and  $I_{0.75}$  treatments, might be because of the crop may not experience water stress under  $I_{1.0}$  and  $I_{0.75}$  treatments significantly. In conformity to this results, Jaybhaye and Mukherjee (2020) reported that water stress was not found under  $I_{1.0}$  and  $I_{0.75}$  treatments during the

study of leaf water potential in broccoli crop.

AET was estimated and water use efficiency (WUE) values were also computed ( $WUE_{\text{watbal}}$ ) by water balance approach. The efficiency of applied water ( $WUE_{\text{watbal}}$ ) reached at maximum level of 12.61 kg/m<sup>3</sup> for applied water of 92.7 mm under  $I_{0.75}$ . Crop water use in the  $I_{1.0}$  irrigation regime was more (117.7 mm) and WUE of that was less (11.08 kg/m<sup>3</sup>). The higher  $ET_c$  and the lower WUE in the  $I_{1.0}$ , may be due to an increase in the 'E' component (evaporation of crop) under frequently irrigation, relative with the 'E' losses under no frequent irrigation of the yield response in the present experiment. The relatively high water use rates are associated with the high frequency of irrigation required for the crop establishment and at the time of high E demand period. The non-frequent irrigation/ deficit irrigation or use of mulching should drastically reduce the need of water for wetting the whole soil surface by irrigation, and thus, reduce the irrigation needs as well as ET of broccoli crop during the early crop growth stages.

Irrespective of water saving techniques (WST), maximum (12.61 kg/m<sup>3</sup>)  $WUE_{\text{watbal}}$  was recorded under  $I_{0.75}$  irrigation regime and it was declined by 12 and 7 % respectively under  $I_{1.0}$  and both  $I_{0.50}$  and  $I_{0.25}$  (Table 1). In general, the application of irrigation enhanced crop yield. However, after some threshold limits, the increase in yield is not proportional to increases amount of irrigation or magnitude of AET. Thus, proportional yield may decrease (Kang et al., 2002). At lower IW/CPE ratio might be decreases cell turgidity, low opening of stomata, which are finally affecting on partitioning of photosynthates to sink and it may because of variations in stomatal density are attribute to the total WUE (Salimath et al., 2023). This was a probable reason for recorded maximum WUE under moderate soil water status ( $I_{0.75}$ ) though quick drying of surface soil caused a rapid reduction in the rate of evaporation, transpiration rate remained unaffected for a long time (Mukherjee et al., 2012) and hence, the broccoli crop was may not face soil moisture stress physiologically in its period under  $I_{0.75}$  treatment; in general, leaf water potential value increased with increasing water stress (at normal climatic condition) but a negligible difference in leaf water

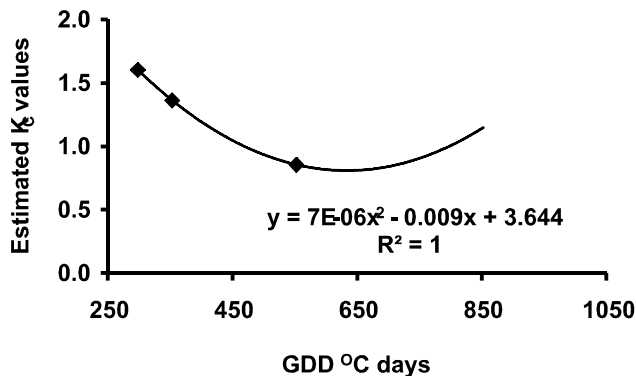


Fig. 4. Relationship between the water depletion crop coefficients-GDD for broccoli under  $I_{1.00}$  irrigation regime

Table 1. Net head yield and water use efficiency (WUE) corresponding to each treatment and net head length and width

Treatments	$ET_c$ fulfilment percentage for each treatment (%)	Water supply (irrigation+rainfall) (mm)	$WUE_{\text{watbal}}$ (kg/m <sup>3</sup> )	Net head yield (kg/ha)	Net head length (cm)	Net head width (cm)
$I_{1.00}$	100	117.7	11.08	15173	11.78	14.77
$I_{0.75}$	75	92.7	12.61	14300	11.11	13.71
$I_{0.50}$	50	92.7	11.69	12218	10.10	11.90
$I_{0.25}$	25	55.2	11.69	9822	9.67	12.01
CD (p=0.05)				1329	1.01	NS

potential value of broccoli was recorded (3 %) in between  $I_{1,0}$  and  $I_{0.75}$  irrigation regimes (Jaybhaye and Mukherjee 2020). Thus, yield of broccoli is not affected more by under minor/moderate soil water stress i.e. irrigation regimes ( $I_{0.75}$ ) resulting in high WUE. This is in agreement with the findings of Lopez-Urrea et al. (2009) for broccoli.

Among the treatments, statistically significant differences were observed in the net head length of broccoli heads (NHL) and no statistically significant differences were noted in the width of broccoli heads (NHW) (Table 1). Irrespective of irrigation regimes and water saving techniques, during the first year of experimentation, the net head length (NHL) was 11.83 cm, which decreased by 20% in 2017-18. It might be due to rainfall immediate after transplanting fevers attack *Lepidoptera* spp. pest; Cutworm (*Agrotis ipsilon* Hufnagel) and also, due to consequently, more attack of (the pest complex recorded on the broccoli crop viz., Leaf webber (*Crociodolomia binotalis* Zeller), beet army worm (*Spodoptera exigua*), Cabbage looper or Leaf eating caterpillar (*Trichoplusia ni* Hübner) and Cross-striped cabbage worm (*Evergestis rimosalis*) during the whole crop growing season of the second year. Furthermore, it might be due of the variation in environmental condition, which is supportive to the statement. The most frequently recharging to the root zone profile of the broccoli crop with irrigation water ( $I_{1,0}$ ) produced the highest NHL (11.78 cm), which was decreased significantly by 6, 14 and 18 % with decrease in status of soil water respectively under  $I_{0.75}$ ,  $I_{0.50}$  and  $I_{0.25}$  treatments. In general, though there was no significant statistical difference between NHL under different irrigation regimes, NHL shows a similar pattern to NHL. The treatments with the least applied water had the lowest NHL and NHW, and this is in agreement with the findings of Lopez-Urrea et al. (2009) for broccoli.

### CONCLUSION

The planting time, application of irrigation levels/irrigation time and water saving techniques significantly affected evapotranspiration, growth and yield of headed broccoli. As the application of water increases, the  $ET_c$ , WUE and yield increased to some extent and thereafter, it decreases; therefore, we tried to quantify the application of water. Net head yield-supplied water relationship, suggested that for obtaining maximum net head yield may require applications of 118 mm water and maximum WUE may require 93 mm of water, while net head yield was found at par under treatment of applied 118 and 93 mm water. The measured  $K_c$  values were higher by 26%, 28% and 48% during initial (rosette development), mid (heading) and end (maturity) stages, respectively compare to the calculated FAO- $K_c$  value of headed broccoli in hot and sub-humid climate. Therefore, it is

recommended that application of 25 % more irrigation to headed broccoli over reference crop evapotranspiration under Indian Hot and Sub-Humid Climatic condition for obtaining economical optimum net head yield.

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# Analysis of Bitter Gourd Hybrids for Yield Traits by using Correlation and Path Coefficient Analysis

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**Abstract:** The correlation and path coefficients of 12 growth and yield parameters in bitter gourd was carried out at the Vegetable Experimental Farm, College of Horticulture, Dr. YSR Horticultural University, Venkataramannagudem, Andhra Pradesh. For all traits, genotypic correlations were stronger than phenotypic correlations, suggesting innate relationships between various attributes. Captivatingly, at both the phenotypic and genotypic levels, yield per plant showed substantial positive associations with plant height, days to 1<sup>st</sup> male flower appearance, number of fruits per plant, average fruit weight, fruit length, fruit diameter, and internodal length and seed per fruit. The results of the path coefficient analysis showed that traits significantly and directly improved fruit yield are number of fruits per plant, plant height, days to 1<sup>st</sup> female flower appearance, sex ratio and fruit diameter. This implies that increasing fruit yield per plant in bitter gourd might be achieved by direct selection focused on these traits.

**Keywords:** Causation factors, Sex ratio, Fruit yield, *Momordica charantia* L.

Bitter gourd, botanically, *Momordica charantia* L., is a well-known herbaceous vine from the Cucurbitaceae family. *Momordica* is a genus of about 45 species, the majority of which are located in Africa (Mabberley 2017). Bitter gourd is a popular "tropical and subtropical" commercially important vegetable crop (Singh et al., 2013). The leading bitter gourd producing states are Maharashtra, Uttar Pradesh, Gujarat, Rajasthan, Punjab, Tamil Nadu, Karnataka, Kerala, Andhra Pradesh, West Bengal, Odisha, Assam and Bihar (NHB Database 2020-21). Yield is a complex trait heavily influenced by the environment, and relying solely on yield for selection may have limitations. On the other hand, yield component traits are less intricate in terms of inheritance and are influenced to a lesser degree by the environment. Consequently, effective improvement in yield can be achieved by selecting various yield component traits that exhibit associations among themselves and with yield. In the current study, phenotypic and genotypic correlations were utilized to ascertain the direct and indirect effects of both yield and yield-contributing characters in the selection of superior cross combinations among bitter gourd genotypes.

## MATERIAL AND METHODS

The experiment was conducted at the Vegetable Experimental farm of the Department of Vegetable Science at Dr. Y.S.R. Horticultural University in V.R. Gudem, West Godavari, Andhra Pradesh during summer and *kharif* seasons of 2022. The 7 parents, 21 hybrids and commercial checks (Pragathi and Monarch) were evaluated in a

randomized complete block design with three replications. The location, situated at an elevation of 34 meters (112 feet) above sea level, falls within Agro-climatic zone 10, characterized by a humid East Coast Plain and Hills (Krishna-Godavari zone) climate, with an average annual rainfall of 900 mm. The region experiences hot and humid summers and pleasant winters. The spacing adopted between row to row and plant to plant is 1m x 1m. Recommended package of practices were followed to raise a healthy crop. Observations were recorded on 12 traits in bitter gourd, with 5 randomly selected plants from each parent and cross in each replication.

**Statistical analysis:** Statistical analysis was conducted using the SPAR-I software. Path analysis was carried out according to the approach proposed by Dewey and Lu (1959).

### Coefficients of Correlation

#### Phenotypic coefficient of correlation:

$$r_{py} = \frac{V_{pxy}}{\sqrt{V_{px} V_{py}}}$$

$V_{pxy}$  = Phenotypic covariance between X and Y

$V_{px}$  = Phenotypic variance of X

$V_{py}$  = Phenotypic variance of Y

#### Genotypic coefficient of correlation: $r_{gy} = \frac{V_{gxy}}{\sqrt{V_{gx} V_{gy}}}$

**Path coefficient analysis:** Path coefficient analysis for traits exhibiting significant correlations with yield was conducted using the approach outlined by Dewey and Lu (1959). The path coefficients were derived through the simultaneous selection of equations that articulate the fundamental

connection between genotypic correlation ( $r$ ) and path coefficient ( $P$ ).

$$r_{14} = P_{14} + P_{24}r_{12} + P_{34}r_{13}$$

$$r_{24} = P_{14}r_{21} + P_{24} + P_{34}r_{23}$$

$$r_{34} = P_{14}r_{31} + P_{24}r_{32} + P_{34}$$

where,  $r_{14}$ ,  $r_{24}$  and  $r_{34}$  are genotypic correlations of component characters with yield (dependent variable) and  $r_{12}$ ,  $r_{13}$  and  $r_{23}$  are the genotypic correlations among component characters (independent variables). The direct effects were calculated by the following set of equations:

$$P_{14} = C_{11}r_{14} + C_{12}r_{24} + C_{13}r_{34}$$

$$P_{24} = C_{21}r_{14} + C_{22}r_{24} + C_{23}r_{34}$$

$$P_{34} = C_{31}r_{14} + C_{32}r_{24} + C_{33}r_{34}$$

where,  $C_{11}$ ,  $C_{22}$ ,  $C_{23}$  and  $C_{33}$  are constants and

$r_{12}P_{24}$ ,  $r_{13}P_{34}$ ,  $r_{21}P_{14}$ ,  $r_{23}P_{34}$ ,  $r_{31}P_{14}$ ,  $r_{32}P_{24}$  are indirect effects.

## RESULTS AND DISCUSSION

**Correlation analysis:** The observed associations are predominantly impacted by genetic variables, as evidenced by the fact that genotypic correlations are generally greater than phenotypic correlations. At the genotypic and phenotypic levels, there was a significant positive connection between plant height and yield per plant. The internodal length, fruit diameter, and average fruit weight all showed significant positive correlations with this characteristic (Table 1, 2). There may be a chance for these traits to be selected for at the same time based on the concurrent growth in one attribute that influences another. In terms of phenotypic

relationships, plant height and fruit number per plant showed a strong positive correlation suggesting that advantageous environmental factors as well as genes have a role in the relationship between this feature and fruit output. The yield per plant and seed per fruit showed a strong positive connection with internodal length. The literature on bitter gourds has demonstrated a consistent pattern of positive associations between vine length, internodal length, and fruit yield. Earlier researcher also observed similar trends (Kumari et al., 2015, Durga et al., 2017, Vivek et al., 2018, Alekar et al., 2019).

The characteristic days to first male flower appearance showed a negative association with fruit length and yield per plant. On the other hand, there was a non-significant negative relationship found between days to first female flower appearance and fruit length, average fruit weight, sex ratio and yield per plant. This implies that choosing fruit production based on when male and female flowers bloom may not be a reliable method. When it came to days to first picking at the genotypic and phenotypic levels, there was a non-significant negative connection with average fruit weight and a significant negative correlation with sex ratio. In bitter gourd, Talukder et al. (2018), Alekar et al. (2019) and Triveni et al. (2021) have also reported significant negative correlation between days to the first male and female flower appearance and fruit yield, demonstrating the consistency of these observations across different studies.

At the phenotypic and genotypic levels, there was a strong and positive correlation between the number of fruits

**Table 1.** Genotypic correlations among fruit yield and yield contributing characters in bitter gourd

Character	Plant height	Day to 1 <sup>st</sup> male flower appearance	Day to 1 <sup>st</sup> female flower appearance	Days to 1 <sup>st</sup> picking	Number of fruits per plant	Average fruit weight	Fruit length	Fruit diameter	Internodal length	Seed per fruit	Sex ratio	Yield per plant
Plant height	1.00	0.202	-0.228	0.110	0.191	0.278**	-0.348**	0.411**	0.411**	0.107	-0.332**	0.338**
Day to 1 <sup>st</sup> male flower appearance		1.00	0.609**	0.027	0.043	0.485**	-0.046	0.154	0.148	0.193	0.410**	0.407**
Day to 1 <sup>st</sup> female flower appearance			1.00	0.042	0.017	-0.071	-0.154	0.053	0.056	0.189	-0.029	-0.091
Days to 1 <sup>st</sup> picking				1.00	0.235**	-0.001	0.173	0.209*	0.208*	0.144	-0.343*	0.066
Number of fruits per plant					1.00	0.112	0.163	0.307**	0.303**	0.111	-0.550**	0.492**
Average fruit weight						1.00	0.376**	0.240*	0.239	0.417**	-0.695**	0.920**
Fruit length							1.00	0.085	0.083	0.520***	-0.398	0.386**
Fruit diameter								1.00	0.007**	0.282*	-0.339*	0.330**
Internodal length									1.00	0.280*	-0.339*	0.328**
Seed per fruit										1.00	-0.349*	0.404**
Sex ratio											1.00	-0.807**
Yield per plant												1.00

\*Significant at 5% level, \*\*significant at 1% level.

per plant and yield per plant, fruit diameter, and internodal length. This suggests that selecting for fruit the number per plant would be very advantageous. At both the phenotypic and genotypic levels, average fruit weight showed similarly strong and positive associations with yield, fruit length, and seeds per fruit, suggesting that selecting for fruit weight would also increase yield. Fruit length showed a strong positive connection with the number of seeds per fruit, yield per plant at the genotypic and phenotypic levels. Similarly, at

both the phenotypic and genotypic levels, fruit diameter exhibited a strong and positive relationship to both yield per plant and the number of seeds per fruit. The consistency of these results across various studies has been confirmed by earlier studies in bitter melon (Gupta et al., 2015, Vivek et al., 2018, Triveni et al., 2021). There was similar associations of fruit number, average fruit weight, fruit length, and diameter with yield and other fruit characteristics in bitter melon. The strong and positive association was between the seeds per

**Table 2.** Phenotypic correlations among fruit yield and yield contributing characters in bitter melon

Character	Plant height	Day to 1 <sup>st</sup> male flower appearance	Day to 1 <sup>st</sup> female flower appearance	Days to 1 <sup>st</sup> picking	Number of fruits per plant	Average fruit weight	Fruit length	Fruit diameter	Internodal length	Seed per fruit	Sex ratio	Yield per plant
Plant height	1.00	0.194	-0.218	0.110	0.187	0.276*	-0.344*	0.405**	0.405**	0.103	-0.327*	0.334**
Day to 1 <sup>st</sup> male flower appearance		1.00	0.559***	0.033	0.048	0.470**	-0.046	0.145	0.144	0.188	0.398**	0.396**
Day to 1 <sup>st</sup> female flower appearance			1.00	0.033	0.022	-0.067	-0.150	0.052	0.053	0.179	0.029	-0.089
Days to 1 <sup>st</sup> picking				1.00	0.227**	-0.001	0.175	0.201*	0.202*	0.141	-0.333*	0.065
Number of fruits per plant					1.00	0.111	0.159	0.302**	0.301**	0.109	-0.545**	0.487**
Average fruit weight						1.00	0.374**	0.238	0.237*	0.416**	-0.693*	0.918**
Fruit length							1.00	0.084	0.083	0.517**	-0.396*	0.384**
Fruit diameter								1.00	0.001	0.279*	-0.338*	0.328**
Internodal length									1.00	0.279*	-0.337*	0.327**
Seed per fruit										1.00	-0.348*	0.403**
Sex ratio											1.00	-0.805**
Yield per plant												-0.069

\*Significant at 5% level, \*\*significant at 1% level.

**Table 3.** Path correlations (Direct and indirect effects) among fruit yield and yield contributing characters in bitter melon

Character	Plant height	Day to 1 <sup>st</sup> male flower appearance	Day to 1 <sup>st</sup> female flower appearance	Days to 1 <sup>st</sup> picking	Number of fruits per plant	Average fruit weight	Fruit length	Fruit diameter	Internodal length	Seed per fruit	Sex ratio	Yield per plant
Plant height	0.2000	0.0404	-0.0456	0.0221	0.0382	0.0557	-0.0696	0.0822	0.0821	0.0213	-0.0663	0.3383**
Day to 1 <sup>st</sup> male flower appearance	-0.0371	-0.1837	-0.1119	-0.0049	-0.0079	-0.0891	0.0085	-0.0283	-0.0273	-0.0354	0.0753	0.4068**
Day to 1 <sup>st</sup> female flower appearance	-0.0508	0.1357	0.2229	0.0093	0.0038	-0.0158	-0.0342	0.0117	0.0124	0.0421	0.0065	-0.0913
Days to 1 <sup>st</sup> picking	0.0008	0.0002	0.0003	0.0074	0.0017	0.000	0.0013	0.0015	0.0015	0.0011	-0.0025	0.0655
Number of fruits per plant	0.0796	0.0179	0.007	0.0978	0.4161	0.0466	0.0677	0.1278	0.1261	0.0463	-0.2288	0.4919**
Average fruit weight	0.299	0.521	-0.0759	0.0009	0.1202	0.0740	0.4037	0.2574	0.2564	0.4478	-0.7465	0.9203**
Fruit length	-0.0325	-0.0043	-0.0143	0.0161	0.0152	0.0351	0.0932	0.0079	0.0077	0.0485	-0.0371	0.3861**
Fruit diameter	-0.0495	-0.0185	-0.0063	0.0251	0.0369	0.0288	0.0102	0.1204	0.1212	0.0339	-0.0408	0.3303**
Internodal length	0.0467	0.0169	0.0063	-0.0237	-0.0345	-0.0272	0.0094	0.1146	0.1138	0.0319	0.0385	0.3277**
Seed per fruit	-0.0103	-0.0186	-0.0182	-0.0138	-0.0107	-0.0402	0.0501	0.0271	-0.027	-0.0963	0.0336	0.4041**
Sex ratio	-0.0404	0.0499	-0.0035	-0.0418	-0.067	-0.0847	-0.0485	-0.0413	-0.0412	-0.0425	0.1218	-0.8074**

\*Significant at 5% level, \*\*significant at 1% level.

fruit and yield per plant. The results of the correlation study showed that there was a strong negative association between the sex ratio and fruit yield per plant. Radha et al. (2015) and Triveni et al. (2021) revealed similar results in bitter gourd.

**Path coefficient analysis:** Plant height showed a strong

positive association and positive direct influence with yield per plant and also had favourable indirect effects on the number of days until the first male flower appeared, the number of days until the first fruit was picked, the average fruit weight, the fruit diameter, internodal length, and number of seeds per fruit. Thus, this characteristic showed a

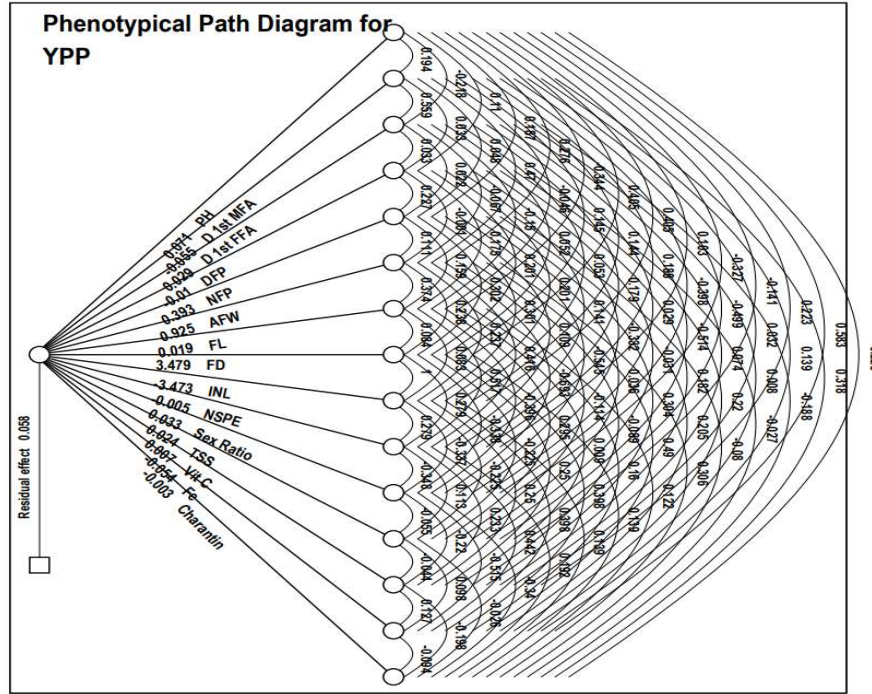


Fig. 1. Phenotypical path diagram for yield per plant (kg)

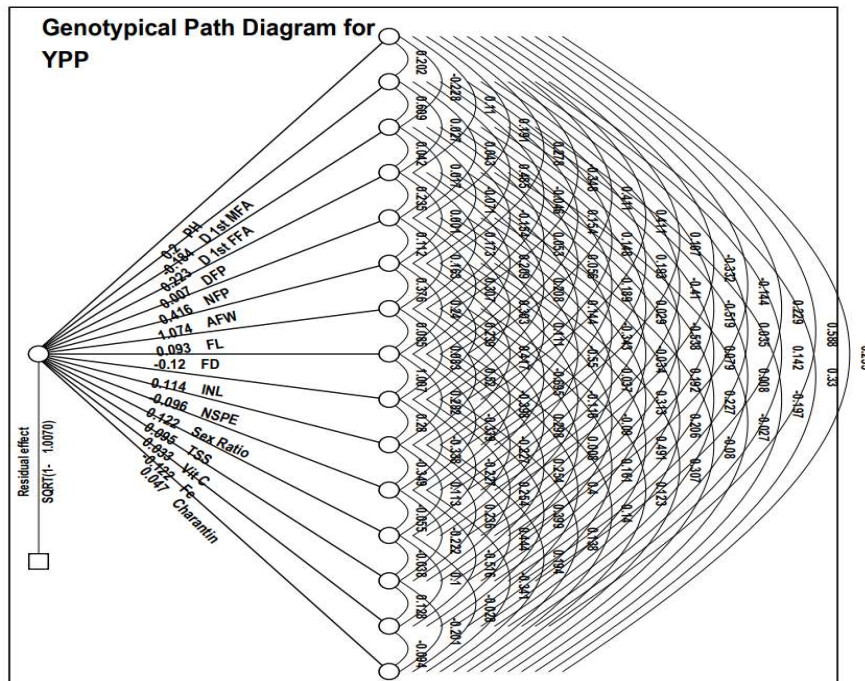


Fig. 2. Genotypical path diagram for yield per plant (kg).



significant beneficial direct influence as well as a positive connection with yield per plant. Likewise, there was a substantial positive correlation and a positive direct effect between internodal length and the dependent variable, which is yield per plant.

The parameter days to first male flower appearance showed a strong positive correlation and a negative direct influence on yield per plant. Plant height, days to first female appearance, days to first harvesting, number of fruits per plant, average fruit weight, fruit diameter, and internodal length were among the other indirect effects that demonstrated unfavourable results. On the other hand, there was a non-significant negative correlation and a positive direct impact between days to first female flower appearance and yield per plant. Additionally, it revealed detrimental indirect impacts via fruit length, average fruit weight, and plant height. The days to first male and female flower appearance showed a negative connection with yield per plant and a positive direct influence. The days to first picking showed a non-significant positive correlation and a positive direct impact at the genotypic and phenotypic levels, respectively.

There was a substantial positive correlation and positive direct impact between the number of fruits per plant and the yield per plant. The plant height, days to first male flower emergence, average fruit weight, internodal length, fruit length, fruit diameter, and seed per fruit all showed positive indirect effects for this characteristic. Similarly, yield per plant was positively correlated and positively affected directly by average fruit weight. Additionally, plant height, days to first male flower emergence, number of fruits per plant, fruit length, and fruit diameter all had a positive indirect impact for this characteristic. There was positive association between the average fruit weight and the number of fruits per plant, as well as a favourable direct influence on yield per plant.

Similarly, there was a substantial positive correlation and positive immediate effect between fruit length and yield per plant. The days to first picking, number of fruits per plant, average fruit weight, fruit diameter, internodal length, and number of seeds per fruit also shown favourable indirect effects. Fruit diameter similarly showed a substantial positive association and positive direct influence with yield per plant. Additionally, this trait showed positive indirect impacts in the following metrics: average fruit weight, internodal length, days to first picking, number of fruits per plant, and seed per fruit.

The number of seeds per fruit showed a strong positive association and a negative direct impact with the yield per plant. With the exception of fruit diameter, which showed a positive influence, this feature showed negative indirect

effects for every trait that was the subject of the analysis. Sex ratio, on the other hand, had a strongly negative association and a positive direct impact with respect to yield per plant. There was also adverse indirect impacts on yield for all characteristics, with the exception of days to male flower appearance.

The average fruit weight was found to have the highest positive direct effect when the path coefficient analysis of different characters that contribute to fruit yield per plant was performed. This was followed by the number of fruits per plant, plant height, days to female flower appearance, fruit length and diameter, internodal length, and sex ratio. It is desirable to choose direct selection based on features that exhibit strong positive direct effects. Studies on bitter gourd in earlier research are consistent with these results (Dubey and Maurya 2013, Pathak et al., 2014, Khan et al., 2015, Singh et al., 2015, Jatav et al., 2016, Alekar et al., 2019 and Triveni et al., 2021).

## CONCLUSIONS

The genotypic correlations will exhibit higher magnitudes compared to their corresponding phenotypic correlations for most traits, suggesting that genotypes are superior, but their expression is diminished under environmental influence. Fruit yield displayed significant and positive correlations with plant height, number of fruits per plant, average fruit weight, fruit length, fruit diameter, internodal length, and seed per fruit. Path analysis further revealed that the traits viz., number of fruits per plant, plant height, days to 1<sup>st</sup> female flower appearance, sex ratio and fruit diameter exerted a direct positive effect on yield, emphasizing the importance of considering these traits collectively for enhancing yield in bitter gourd cultivation.

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Received 27 September, 2024; Accepted 30 December, 2024



# Elimination of Hard Lumps in Malbhog Banana (*Musa*, AAB) by Application of Growth Regulators and Micronutrients

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**Abstract:** Combined effect of different plant growth regulators and micronutrients on incidence of hard lump of Malbhog banana was observed at Uttar Banga Krishi Vishwavidyalaya, Pundibari, West Bengal for main and ratoon crop. Combinations of GA<sub>3</sub> (@0, 50, 100 ppm) and 2,4-D (@ 0, 50, 100 ppm) as foliar spray along with borax (soil application @ 0, 1, 2 g/plant; foliar application @ 0, 0.25, 0.50 %) and zinc sulphate heptahydrate (soil application @ 0, 2, 4 g/plant and foliar application @ 0, 0.25, 0.50 %) were used as the sources of boron and zinc respectively at 3rd, 5th and 7th months after planting. The hard lumps separated from the ripe banana fruits to record different observations. Colourless or brownish coloured, large sized lumps were detected with high fruit pressure from most of the control treatments (no growth regulators and micronutrients). Most soft fruits with minimum or no lumps recorded in GA<sub>3</sub> and 2,4-D (both @100ppm). Among the soil application of micronutrients 1g borax with 2g zinc sulphate/plant application and foliar application of both the borax and zinc sulphate @ 0.25% resulted best with respect to fruit pressure and less incidence of lumps.

**Keywords:** GA<sub>3</sub>, 2,4-D, Boron, Zinc, Hard lump, Malbhog banana

Banana (*Musa* sp.) is one of the most important commercial fruit crops of India cultivated in 959 thousand ha area is under banana cultivation and producing the yield of 35131 thousand MT during the year 2021-22 (Keelery 2023). Dwarf Cavendish, Kabuli, Marthaman, Champa, Kanthalie are most important varieties which are cultivated throughout India (Amini et al., 2019; Deb and Sinha 2024). Malbhog or Rashthali (*Musa*, AAB) is one of the popular cultivars of northern part of West Bengal and North East India (Kumar et al., 2020, Subba et al., 2024).

The wide range of banana cultivation is being attempted by the growers of Bengal, especially of Terai region with the increasing trend Terai soils are acidic in reaction (pH 4.8-6.5) and is having high rainfall, leading to high leaching loss of nutrients. Therefore, important micronutrients are not available to the plants according to their requirement (Bhowmick et al., 2017). Among different cultivation problems formation of hard lumps within the fruit pulp is one of the serious problems of Malbhog banana, the choicest banana variety of northern part of West Bengal. Very fresh and healthy fruits may also contain fair proportion of hard lumps within the fruit pulp due to which the ripe fruits become unsuitable for consumption. Hard lumps are the dry mass of cells often mixed with pulp which results the banana fruit not suitable for consumption in severe cases. This problem is supposed to be associated with imbalance of plant growth hormones and micronutrients during the finger development

and maturity within the fruit tissue (Gonmei et al., 2022) which is very similar to dead tissue development in many other fruits (Basra 2000, Davies 2013) and uneven growth of tissues in same organ (Singh et al., 2021). However, no information on elimination of hard lump formation in Malbhog banana is lacking. The current research aimed to study the impact of application of some growth regulators and micronutrients on hard lumps of formation of Malbhog banana.

## MATERIAL AND METHODS

**Location site:** The experiment was conducted at the Farm of Uttar Banga Krishi Vishwavidyalaya, Pundibari, Cooch Behar, West Bengal. The site is located 26°19' N latitude and 89°29' E longitude and having an altitude of 43 meters above mean sea level. The soil of the experimental field was sandy loam in nature, coarse in texture having poor water holding capacity with low pH (5.23). The climatic condition of the terai zone is characterized by high rainfall (above 3000 mm annually), high humidity, moderate temperature, prolonged winter with high residual soil moisture.

**Planting and after care:** Sword suckers of banana plants (variety Malbhog) having average weight of 2.5 kg were planted in well prepared field with spacing of 2 x 2 m during April' 2021. 150 kg nitrogen, 150 kg P<sub>2</sub>O<sub>5</sub> and 200 kg K<sub>2</sub>O per ha were applied in three split doses. Need based application of pesticides and fungicides was followed for main crop as well as ratoon crop.

**Experimental details:** The treatment combinations of growth regulators (GA<sub>3</sub> and 2,4-D) and micronutrients (borax i.e. Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, 10 H<sub>2</sub>O) as source of boron and zinc sulphate i.e. ZnSO<sub>4</sub>, 7H<sub>2</sub>O as source of zinc) were used (Table 1, 2). The experiment was laid out in randomized block design (RBD) with 9 treatments of growth regulators and micronutrient combinations each replicated thrice. Soil and foliar application of micronutrients were combined with foliar application of growth regulators.

**Application of growth regulators and micronutrients:** Growth regulators (GA<sub>3</sub> and 2,4-D) were applied separately (keeping 2-3 days interval) at 5 and 7 months after planting and after flowering during the morning hours of sunny days in an amount of 500 ml/plant in respective doses. Micronutrients were applied separately (keeping 2-3 days interval) for foliar application adjusting the pH 7.0 at 3, 5 and 7 months after planting during the morning hours of sunny days in an amount of 500 ml/plant in respective doses. But in case of soil application both micronutrients were applied on same day.

**Observations:** Harvesting of fruit was done at fully mature stage and fruits were brought to the laboratory (Department of Horticulture and Postharvest Technology, Institute of Agriculture, Visva-Bharati) After ripening, fruits were peeled

out and smashed lightly with two fingers to find out the lumps. Incidence of lumps were expressed in percentage. Three different coloured of lumps were observed under different treatments like brownish, yellow and self-colored (colour of pulp). Positions of lumps were classified according to placental region, inner mesocarp and outer mesocarp. The length of separated lumps were measured with a centimeter scale. The weights of separated lumps were measured in gram. Toughness or texture of ripe fruit was measured with the help of fruit pressure tester (penetrometer) using the penetration ring nut radius of 4.0 mm. Calibration was expressed in terms of kg per square centimeter.

**Statistical analysis:** Data collected from the field experiment were subjected to statistical analysis using Statistical Package for Social Sciences (IBM SPSS Version 27.0).

## RESULTS AND DISCUSSION

**Incidence of hard lump formation:** Incidence of hard lump formation (Table 3) was highest (27.9 and 24.1 % in soil and foliar application of micronutrients, respectively) under T<sub>1</sub> (control or having no growth regulators and micronutrients) whereas it is minimum as 5.5 and 5.6 % under E<sub>1</sub>T<sub>8</sub> (GA<sub>3</sub> and 2,4-D both @100ppm along with soil application of borax @

**Table 1.** Treatment combinations of growth regulators with soil application of micronutrients for first set of experiment (E<sub>1</sub>)

Treatments	Growth regulators	Micro nutrients used (per plant)
E <sub>1</sub> T <sub>1</sub>	No application (Control)	No application (Control)
E <sub>1</sub> T <sub>2</sub>	No application	Borax @ 1g + Zinc sulphate @ 2g
E <sub>1</sub> T <sub>3</sub>	No application	Borax @ 2g + Zinc sulphate @ 4g
E <sub>1</sub> T <sub>4</sub>	GA <sub>3</sub> @ 50ppm + 2,4-D @ 50ppm	No application
E <sub>1</sub> T <sub>5</sub>	GA <sub>3</sub> @ 50ppm + 2,4-D @ 50ppm	Borax @ 1g + Zinc sulphate @ 2g
E <sub>1</sub> T <sub>6</sub>	GA <sub>3</sub> @ 50ppm + 2,4-D @ 50ppm	Borax @ 2g + Zinc sulphate @ 4g
E <sub>1</sub> T <sub>7</sub>	GA <sub>3</sub> @ 100ppm + 2,4-D @ 100ppm	No application
E <sub>1</sub> T <sub>8</sub>	GA <sub>3</sub> @ 100ppm + 2,4-D @ 100ppm	Borax @ 1g + Zinc sulphate @ 2g
E <sub>1</sub> T <sub>9</sub>	GA <sub>3</sub> @ 100ppm + 2,4-D @ 100ppm	Borax @ 2g + Zinc sulphate @ 4g

**Table 2.** Treatment combinations of growth regulators with foliar application of micronutrients for second set of experiment (E<sub>2</sub>)

Treatments	Growth regulators used	Micro nutrients used
E <sub>2</sub> T <sub>1</sub>	No application (Control)	No application (Control)
E <sub>2</sub> T <sub>2</sub>	No application	Borax @ 0.25 % + Zinc sulphate @ 0.25 %
E <sub>2</sub> T <sub>3</sub>	No application	Borax @ 0.50 % + Zinc sulphate @ 0.50 %
E <sub>2</sub> T <sub>4</sub>	GA <sub>3</sub> @ 50ppm + 2,4-D @ 50ppm	No application
E <sub>2</sub> T <sub>5</sub>	GA <sub>3</sub> @ 50ppm + 2,4-D @ 50ppm	Borax @ 0.25 % + Zinc sulphate @ 0.25 %
E <sub>2</sub> T <sub>6</sub>	GA <sub>3</sub> @ 50ppm + 2,4-D @ 50ppm	Borax @ 0.50 % + Zinc sulphate @ 0.50 %
E <sub>2</sub> T <sub>7</sub>	GA <sub>3</sub> @ 100ppm + 2,4-D @ 100ppm	No application
E <sub>2</sub> T <sub>8</sub>	GA <sub>3</sub> @ 100ppm + 2,4-D @ 100ppm	Borax @ 0.25 % + Zinc sulphate @ 0.25 %
E <sub>2</sub> T <sub>9</sub>	GA <sub>3</sub> @ 100ppm + 2,4-D @ 100ppm	Borax @ 0.50 % + Zinc sulphate @ 0.50 %

1g and Zinc sulphate @2g/plant) and E<sub>2</sub>T<sub>8</sub> (GA<sub>3</sub> and 2,4-D @100ppm and foliar application of borax @ 0.25 % + Zinc sulphate @ 0.25 %). The formation of hard lumps was decreased with increased in the dose of growth regulators. In both the cases of soil and foliar application of micronutrients, moderate dose (Borax @ 0.25 % + Zinc sulphate @ 0.25 %) resulted better in terms of lesser incidence of hard lumps. Combination of both the growth regulators and micronutrients resulted lesser incidence of hard lumps in the banana fruit pulp rather it is higher in solo application of growth regulators or micro nutrients. But lowest rate of formation of hard lumps was observed in E<sub>2</sub>T<sub>8</sub> (growth regulators with foliar application of micronutrients). Growth regulators probably played a role in uniformity of cell division and growth of cells within the fruit tissue due to which there was minimum lumps. Besides micronutrients might have helped in better translocation of nutrients to the developing fruit (Gonmei et al, 2022). Lee (2003) revealed that micronutrients particularly zinc and boron have positive role in auxin distribution and proper tissue development in growing fruits, respectively. This might be the cause of the pattern of hard lump formation in the treatments without zinc application. Additionally, incidents of hard lump were lower in ratoon crop than in main crop across the treatments. This might be attributed to the result of application of micronutrients as well as growth regulators which impacted the proper growth and nutrient storage of mother rhizome from which the ratoon plant arisen.

**Colour of hard lumps:** The colour of lumps become lighter from brown to yellow and ultimately self-colored (*i.e.*, the colour of fruit pulp) with increasing the dose of growth regulators (Table 4). There were no such changes in colour

with the increase of the doses of micronutrients. But higher doses of growth regulators played a great role in reducing the intensity of the colour of hard lumps. Growth regulators along with micronutrients might have reduced the formation of narrow minute patches of dead cells within the living cells of fruit tissues which reduced the intensity of the colour of hard lumps in the present experiment (Bauri et al, 2014). Davies (2013) observed that the imbalance in growth regulator distribution may cause the cell death in patches in soft growing tissues and this support present findings. Escalante et al (2018) mentioned the colour variation of banana pulp and browning may be due to imbalance of physiological process.

**Position of hard lumps:** Different positions of hard lumps in ripe banana fruits have been observed in the present experiment (Table 5). The hard lumps have different

**Table 4.** Colour of hard lumps in the ripe banana fruits under different treatment combinations of growth regulators and micronutrients

Treatments	Growth regulators with soil application of micronutrients
E <sub>1</sub> T <sub>1</sub> , E <sub>2</sub> T <sub>1</sub>	Brown
E <sub>1</sub> T <sub>2</sub> , E <sub>2</sub> T <sub>2</sub>	Brown
E <sub>1</sub> T <sub>3</sub>	Yellow
E <sub>1</sub> T <sub>4</sub>	Yellow
E <sub>1</sub> T <sub>5</sub>	Yellow
E <sub>1</sub> T <sub>6</sub>	Yellow, Self-coloured
E <sub>1</sub> T <sub>7</sub>	Self-coloured
E <sub>1</sub> T <sub>8</sub>	Self-coloured
E <sub>1</sub> T <sub>9</sub>	Self-coloured

**Table 3.** Incidence of hard lumps in the ripe banana fruits under different treatment combinations of growth regulators and micronutrients (%)

Treatments	Growth regulators with soil application of micronutrients			Treatments	Growth regulators with foliar application of micronutrients		
	Main crop	Ratoon crop	Pooled		Main crop	Ratoon crop	Pooled
E <sub>1</sub> T <sub>1</sub>	28.6	27.3	27.9	E <sub>2</sub> T <sub>1</sub>	25.5	23.7	24.1
E <sub>1</sub> T <sub>2</sub>	24.9	25.4	25.1	E <sub>2</sub> T <sub>2</sub>	23.2	20.6	21.9
E <sub>1</sub> T <sub>3</sub>	18.3	17.7	18.0	E <sub>2</sub> T <sub>3</sub>	16.7	13.9	15.3
E <sub>1</sub> T <sub>4</sub>	17.2	14.3	15.7	E <sub>2</sub> T <sub>4</sub>	14.2	12.0	13.1
E <sub>1</sub> T <sub>5</sub>	13.8	14.5	14.1	E <sub>2</sub> T <sub>5</sub>	11.3	12.7	12.0
E <sub>1</sub> T <sub>6</sub>	14.7	12.3	13.5	E <sub>2</sub> T <sub>6</sub>	10.5	9.0	9.7
E <sub>1</sub> T <sub>7</sub>	11.3	9.5	10.4	E <sub>2</sub> T <sub>7</sub>	6.7	7.8	7.2
E <sub>1</sub> T <sub>8</sub>	5.8	5.3	5.5	E <sub>2</sub> T <sub>8</sub>	5.1	6.2	5.6
E <sub>1</sub> T <sub>9</sub>	8.7	6.9	7.8	E <sub>2</sub> T <sub>9</sub>	7.9	6.3	7.1
CD (p=0.05)	2.65	2.06	2.31	CD (p=0.05)	2.42	2.34	1.87

positions like core of the placenta, whole placenta, inner mesocarp, outer mesocarp etc. The hard lumps in banana fruits exhibited that the application of growth regulators reduced the scattering of hard lumps. With the increase in the dose of growth regulators resulted in concentric position of lumps near to the placental core of the banana fruits. The control treatment resulted the most scattered position of hard lumps in entire mesocarp and placental core. Growth regulators might have direct effect of proper connective tissues throughout the fruits and entire mesocarp which resulted no such occurrence of hard lumps in the fruit mesocarp.

**Length of hard lumps:** Significantly higher length of hard lumps (Table 6) was in  $T_1$  of both soil and foliar application of micronutrients (2.10 and 2.15 cm, respectively no application of growth regulators and micronutrients (Table 6).  $E_1T_1$  and  $E_2T_1$  have resulted increased growth of hard lumps and thus highest length of lumps have been attained. Conversely,

shortest length of hard lumps was observed under  $T_8$  in both the soil and foliar application of micronutrients (0.78 cm and 0.80 cm, respectively in  $E_1T_8$  and  $E_2T_8$ ). Both the soil and foliar application of micronutrients have shown similar trend and moderate dose of micronutrients has resulted lowest length of hard lumps while the higher dose and no application of micronutrients exhibited greater length of hard lumps. The deficiency of micronutrients as well as profuse use might cause physiological abnormalities that resulted higher length of hard lumps.

**Average weight of hard lumps:** Average weight of hard lumps of banana fruits was maximum (6.07g and 5.65g respectively) in  $E_1T_1$  (growth regulators with soil application of micronutrients) and  $E_2T_1$  (growth regulators with foliar application of micronutrients) though weight of hard lumps were slightly lower under foliar application (*i.e.* second set of experiment or  $E_2$ ) (Table 7). Minimum weight of hard lumps was in  $E_1T_8$  and  $E_2T_8$  (0.94 and 1.01g in soil and foliar

**Table 5.** Position of hard lumps in the ripe banana fruits under different treatment combinations of growth regulators and micronutrients

Treatments	Growth regulators with soil application of micronutrients	Treatments	Growth regulators with foliar application of micronutrients
$E_1T_1$	Placenta, outer and inner mesocarp	$E_2T_1$	Placenta, outer and inner mesocarp
$E_1T_2$	Placenta, outer and inner mesocarp	$E_2T_2$	Outer and inner mesocarp
$E_1T_3$	Outer and inner mesocarp	$E_2T_3$	Outer and inner mesocarp
$E_1T_4$	Outer and inner mesocarp	$E_2T_4$	Outer mesocarp
$E_1T_5$	Inner mesocarp	$E_2T_5$	Outer mesocarp
$E_1T_6$	Inner mesocarp	$E_2T_6$	Inner mesocarp
$E_1T_7$	Total placenta	$E_2T_7$	Core of placenta
$E_1T_8$	Core of placenta	$E_2T_8$	Core of placenta
$E_1T_9$	Core of placenta	$E_2T_9$	Core of placenta

**Table 6.** Length of hard lumps in the ripe banana fruits under different treatment combinations of growth regulators and micronutrients

Treatments	Growth regulators with soil application of micronutrients			Treatments	Growth regulators with foliar application of micronutrients		
	Main crop	Ratoon crop	Pooled		Main crop	Ratoon crop	Pooled
$E_1T_1$	2.16	2.05	2.10	$E_2T_1$	2.21	2.09	2.15
$E_1T_2$	1.85	1.92	1.88	$E_2T_2$	1.80	1.83	1.81
$E_1T_3$	1.53	1.45	1.69	$E_2T_3$	1.62	1.53	1.57
$E_1T_4$	1.95	1.98	1.96	$E_2T_4$	1.61	1.66	1.63
$E_1T_5$	1.21	1.03	1.12	$E_2T_5$	1.12	0.98	1.05
$E_1T_6$	1.21	1.32	1.26	$E_2T_6$	1.05	1.12	1.08
$E_1T_7$	1.07	0.96	1.02	$E_2T_7$	0.78	0.89	0.83
$E_1T_8$	0.81	0.75	0.78	$E_2T_8$	0.77	0.83	0.80
$E_1T_9$	0.96	0.88	0.92	$E_2T_9$	0.93	1.11	1.02
CD (p=0.05)	0.18	0.17	0.18	CD (p=0.05)	0.20	0.21	0.19

application of micronutrients with growth regulators respectively). Higher doses of growth regulators decreased the total amount of lumps in banana fruits. Medium dose of micronutrients (soil application of borax @ 1g and zinc sulphate @2g/plant or foliar application of borax @ 0.25 % and zinc sulphate @ 0.25 %) resulted lower average weight of lumps when no growth regulators were applied while both the medium and high dose of micronutrients resulted more or less similar weight of lumps in banana fruits. Comparing the results of treatment combinations, indicate that addition of micronutrients with growth regulators have markedly reduced the lump weight. The positive role of gibberellic acid and auxins including the synthetic auxins (like 2,4-D) on proper growth of the fruit pulps during growth and development might have reduced the chances of physiological disorder like hardening of fruit pulp ( Basra

2000). Application of micronutrients like zinc might have caused higher synthesis of auxin within the developing fruits that resulted into reduced size of lumps in banana fruit pulp. The reduced weight of lumps by application of gibberellin and 2,4-D as well as the micronutrients is supported by the report of Basra (2000).

**Fruit pressure (kg/cm<sup>2</sup>):** Fruit pressure was statistically maximum (2.81 and 2.82 kg/cm<sup>2</sup>) under both the E<sub>1</sub>T<sub>1</sub> and E<sub>2</sub>T<sub>1</sub> with no application of growth regulators and micronutrients in both set of experiments (Table 8). Application of micronutrients significantly reduced incidence as well as size of the lumps in the banana caused reduced hardness of the banana fruits. Thus the higher dose of growth regulators and medium dose of micronutrients under both the soil and foliar application of micronutrients i.e. E<sub>1</sub>T<sub>8</sub> (GA<sub>3</sub> and 2,4-D both @100ppm along with soil application of borax @

**Table 7.** Average weight (g) of hard lumps in the ripe banana fruits under different treatment combinations of growth regulators and micronutrients

Treatments	Growth regulators with soil application of micronutrients			Treatments	Growth regulators with foliar application of micronutrients		
	Main crop	Ratoon crop	Pooled		Main crop	Ratoon crop	Pooled
E <sub>1</sub> T <sub>1</sub>	6.15	6.00	6.07	E <sub>2</sub> T <sub>1</sub>	5.28	6.03	5.65
E <sub>1</sub> T <sub>2</sub>	5.27	5.03	5.15	E <sub>2</sub> T <sub>2</sub>	2.77	2.89	2.83
E <sub>1</sub> T <sub>3</sub>	4.20	4.32	4.26	E <sub>2</sub> T <sub>3</sub>	3.94	4.16	4.05
E <sub>1</sub> T <sub>4</sub>	4.76	4.51	4.63	E <sub>2</sub> T <sub>4</sub>	3.82	3.65	3.73
E <sub>1</sub> T <sub>5</sub>	2.11	1.93	2.02	E <sub>2</sub> T <sub>5</sub>	2.02	1.86	1.94
E <sub>1</sub> T <sub>6</sub>	3.23	3.36	3.29	E <sub>2</sub> T <sub>6</sub>	2.93	3.14	3.03
E <sub>1</sub> T <sub>7</sub>	2.81	2.70	2.75	E <sub>2</sub> T <sub>7</sub>	2.22	2.41	2.32
E <sub>1</sub> T <sub>8</sub>	0.90	0.99	0.94	E <sub>2</sub> T <sub>8</sub>	1.10	0.92	1.01
E <sub>1</sub> T <sub>9</sub>	1.36	1.48	1.42	E <sub>2</sub> T <sub>9</sub>	1.27	1.35	1.31
CD (p=0.05)	0.41	0.38	0.39	CD (p=0.05)	0.44	0.37	0.42

**Table 8.** Fruit pressure of ripe banana fruits under different treatment combinations of growth regulators and micronutrients (kg/cm<sup>2</sup>)

Treatments	Growth regulators with soil application of micronutrients			Treatments	Growth regulators with foliar application of micronutrients		
	Main crop	Ratoon crop	Pooled		Main crop	Ratoon crop	Pooled
E <sub>1</sub> T <sub>1</sub>	2.78	2.84	2.81	E <sub>2</sub> T <sub>1</sub>	2.80	2.84	2.82
E <sub>1</sub> T <sub>2</sub>	2.66	2.70	2.68	E <sub>2</sub> T <sub>2</sub>	2.73	2.65	2.69
E <sub>1</sub> T <sub>3</sub>	2.67	2.71	2.69	E <sub>2</sub> T <sub>3</sub>	2.62	2.67	2.64
E <sub>1</sub> T <sub>4</sub>	2.44	2.39	2.41	E <sub>2</sub> T <sub>4</sub>	2.46	2.36	2.41
E <sub>1</sub> T <sub>5</sub>	2.32	2.28	2.30	E <sub>2</sub> T <sub>5</sub>	2.32	2.50	2.41
E <sub>1</sub> T <sub>6</sub>	2.53	2.46	2.50	E <sub>2</sub> T <sub>6</sub>	2.03	2.15	2.09
E <sub>1</sub> T <sub>7</sub>	1.90	1.95	1.92	E <sub>2</sub> T <sub>7</sub>	1.89	1.81	1.85
E <sub>1</sub> T <sub>8</sub>	1.69	1.60	1.65	E <sub>2</sub> T <sub>8</sub>	1.62	1.75	1.69
E <sub>1</sub> T <sub>9</sub>	1.68	1.74	1.71	E <sub>2</sub> T <sub>9</sub>	1.83	1.88	1.84
CD (p=0.05)	0.15	0.17	0.14	CD (p=0.05)	0.16	0.18	0.15

1g and Zinc sulphate @2g/plant) and E<sub>2</sub>T<sub>8</sub> (GA<sub>3</sub> and 2,4-D @100ppm and foliar application of borax @ 0.25 % + Zinc sulphate @ 0.25 %) showed the minimum fruit pressure (1.65 and 1.69 kg/cm<sup>2</sup>). The fruit pressure was decreased with increment in the dose of growth regulators and micronutrients. As growth regulators and micronutrients reduced the size and weight of the hard lumps fruit pressure also decreased that mean fruits become softer. Singh *et al.* (2021) has described the positive influence of gibberellins and auxins in proper tissue development during sexual growth in plants and deficiency of such growth regulators may also cause reduced growth by hardening the tissues and dead patch formation. Thus, the deficiency of growth regulators in the present experiment caused increased hardness of lumps in banana fruits under treatments having no growth regulators (control treatments; E<sub>1</sub>T<sub>1</sub> and E<sub>2</sub>T<sub>1</sub>). This has consonance with the statement of Singh et al (2021). Antonio and Augusto (2020) and Wang et al (2024) have also reported higher fruit pressure in banana due to improper physiological advancement of growth and ripening for imbalance of growth regulators and micronutrients.

Sarma et al (2001) reported that pulp hardness might be reduced by application of 2,4-D in higher dose after harvesting in *Musa* (AAB group) cv. Malbhog. The softer pulp of banana free mostly free from hard lumps in the present experiment with the application of growth regulators and micronutrients was due to proper availability of different growth regulators and micronutrients for the fruit pulp tissue development in banana. Banana fruits with no application of micronutrients in the present study exhibited comparatively hard fruits than the micronutrient applied fruits. The present research has also similarity with the findings of Matsumoto et al (2009). Gonçalves and Vitória (2011) reported the deficiency of zinc and boron in papaya caused pulp hardness. McMenemy (2014) described hard lumps in flesh of avocado fruit due to nutritional deficiency and micronutrients in particular.

### CONCLUSIONS

Colourless or brownish, large sized lumps were detected in the banana fruits with higher fruit pressure from most of the control treatments (i.e. having no growth regulators and micronutrient application). Most soft fruits with minimum or no lumps have been recorded under maximum doses of growth regulators. Among the soil application of micronutrients, 1g borax with 2g zinc sulphate per plant application resulted best in respect to fruit pressure and less occurrence of hard lumps in banana. But 0.25% of both the borax and zinc sulphate as foliar application has also shown better result with respect to less occurrence of hard lumps

and softer fruits. Hence, to get banana fruits with minimum hard lumps, GA<sub>3</sub> and 2,4-D (both @100ppm) with borax and zinc sulphate (@1 and 2g/plant as soil application, respectively or 0.25% foliar application of both) can be followed.

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Received 04 October, 2024; Accepted 24 January, 2025



# Performance of Avenue Tree Species Grown Through Seedballs Encapsulated with Varying Soil Media Mixtures in Subtropical Climate of Punjab

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**Abstract:** Seeding an extensive wasteland requires an ideal environment for seeds to germinate in arid ecosystems. This investigation was conducted to study the performance of avenue tree species encapsulated in different media mixtures for their germination potential and growth behavior at Punjab Agricultural University, Ludhiana during 2020-22. Seeds from five avenue tree species (*Cassia fistula*, *Azadirachta indica*, *Schleichera oleosa*, *Pongamia pinnata* and *Putranjiva roxburghii*) were encapsulated. These seedballs were sown on three different dates viz. 15<sup>th</sup> July, 15<sup>th</sup> August and 15<sup>th</sup> September. In all sowing date, *Azadirachta indica* seedballs encapsulated in soil + cocopeat (1:1, v/v) exhibited maximum germination percentage (90, 89.67 and 78 %). *Cassia fistula* seed balls encapsulated in soil + cocopeat germinated earliest (7.35, 9.18 and 11.25 days, respectively) with maximum plant height (39.51, 40.35 and 38.12 cm), higher leaf area (73.11, 75.28 and 71.67 cm<sup>2</sup>) and highest survival percentage (47.33, 50.67 and 41.33%). The seedball encapsulated in mixture of soil + cocopeat (1:1, v/v) created an ideal micro-environmental condition for seed germination for these tree species and can be utilized them for the restoration of degraded landscapes and wastelands.

**Keywords:** Avenue tree, Subtropical climate, Germination, seedball, Soil media mixtures

Today's world is facing extreme climate breakdown. Global warming is one of the factors of climate irregularities causing unfavorable conditions for plant growth. In India, the reduction of natural forest cover was reported around 117 thousand ha (2022), which is equivalent to CO<sub>2</sub> emission of 62.9 MT (Anonymous 2023). This leads the changes in microclimate where the deforestation rate is quite high. One of the best strategies for preventing global warming effect and to sustain the life is afforestation. The success rate of afforestation initiatives is unfortunately impacted by high costs and risk considerations associated with handling tree saplings and providing initial care after planting. Since the seeds in the seed ball technique are shielded from outside stress and predators, it is an efficient method of growing plants from seed (Tamilarasan et al., 2021a). To restore greening of these extensively degraded landscapes by encouraging seeding of perennial and multipurpose tree species, several alternatives have been tested by several researchers that could address the issue of seed germination under natural conditions.

Traditionally, seedball technique was being used to green wastelands by encapsulating healthy mature seeds having hard seed coat within the mixture of clay and soil (Fukuoka 1978). However, today different media like clay, cocopeat, FYM, garden soil and nutrient mixtures can be used for the

formation of the seed balls. Seedballs are used for restoration of extensive barren and unproductive lands with minimal labour and resources. Areas inappropriate for conventional planting can be effectively rejuvenated through seedball dispersal (Ilan et al., 2015). Encapsulating seeds with soil and other media mixtures significantly helps in the protection of seeds from insect-pest and rodents. These media mixture maintain the structure of the seedballs and provide nutrients and required moisture to the seed for better germination and establishment.

A genetic diversity in cultivated plant material and eventually, capacity to adapt to a reintroduction site might be influenced by the methods used in production technique. Applying seed pretreatment especially in seed ball technique to reduce physiological seed dormancy and promote effective seed germination is a standard step in the plant production process (Diaz-Martin et al., 2023). Limited literature is available with respect to identification of suitable tree species for greening degraded lands through seedball technique. Additionally, specific compositions that can enhance the survival and germination of the seeds in uncultivated lands are needed to be investigated. Hence, the present study was planned with the hypothesis that seeds of different indigenous avenue tree species encapsulated with different seed ball soil media mixtures and planted during

different months will likely show effect seed germination, survival and establishment.

### MATERIAL AND METHODS

Seedballs of five avenue tree species ( $S_1$  - *Cassia fistula*,  $S_2$  - *Azadirachta indica*,  $S_3$  - *Schleichera oleosa*,  $S_4$  - *Pongamia pinnata* and  $S_5$  - *Putranjiva roxburghii*) were prepared using five different media mixture viz.  $M_1$  [soil + clay (1:1 v/v)],  $M_2$  [soil + FYM (1:1 v/v)],  $M_3$  [soil + cocopeat (1:1 v/v)],  $M_4$  [soil + cocopeat + clay (4:3:3 v/v)] and  $M_5$  (seed priming with clay). Each seedball contained one seed having diameter of 4 cm.

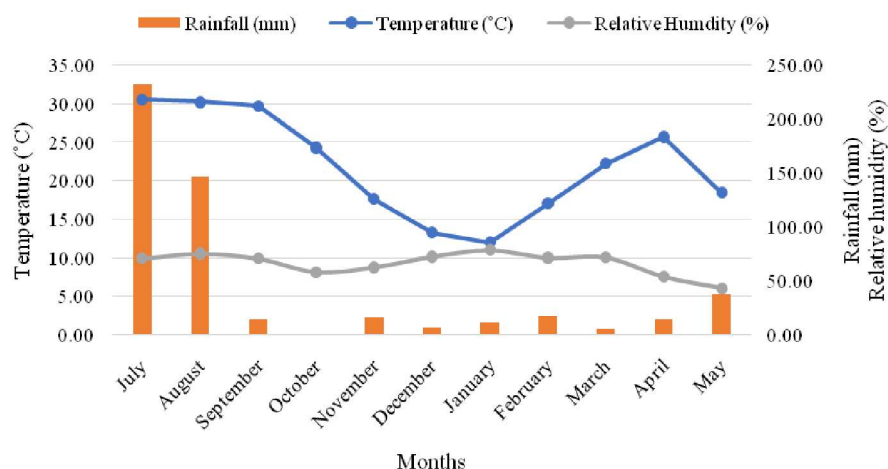
These prepared seedballs were sown in three locations in the PAU campus on three different sowing periods, viz.,  $D_1$  (15<sup>th</sup> July),  $D_2$  (15<sup>th</sup> August) and  $D_3$  (15<sup>th</sup> September) for two consecutive years, i.e. 2020-21 and 2021-22. The experiment was laid out in factorial randomized block design (FRBD) with 25 treatments with three replications. Ten number of seed balls per treatment per replication were taken with total 2250 seedball in experiment. The observations of germination percentage, days to germination was recorded at 10 days of sowing and plant height (cm), number of leaves were observed at 3-month interval while leaf area (cm<sup>2</sup>), stem girth (mm), survival percentage of plants, pH (Jackson 1967) and EC (Jackson 1967) of media were recorded at 9 months to days of sowing. The interaction results of species and media have been discussed to interpret the conclusions out of this investigation. The statistical analysis of the data was conducted in SAS software version 9.0. Further the multiple comparison to separate the treatment means was performed using Duncan's multiple range test (DMRT).

### RESULTS AND DISCUSSION

**Germination parameters:** The germination percentage and days to germination were significantly affected by the

treatments (species and media). In  $D_1$  sowing (15<sup>th</sup> July), the maximum (90.00 %) and the minimum (52.00 %) germination percentage were obtained in  $S_2M_3$  (*Azadirachta indica* grown in soil + cocopeat media) and  $S_3M_2$  (*Schleichera oleosa* grown in soil + FYM media), respectively (Table 1). In  $D_2$  (15<sup>th</sup> August) sowing,  $S_2M_3$  (*Azadirachta indica* in soil + cocopeat media) and  $S_3M_1$  (*Schleichera oleosa* in soil + clay media) exhibited maximum (89.67 %) and minimum (49.67 %) germination percentage, respectively. In  $D_3$  sowing (15<sup>th</sup> September), the maximum (78.00 %) and minimum (42.00 %) germination percentage were in  $S_2M_3$  (*Azadirachta indica* in soil + cocopeat media) and  $S_3M_1$  (*Schleichera oleosa* in soil + clay media), respectively.

In  $D_1$  (15<sup>th</sup> July), earlier germination (7.35 days) was recorded in the treatment  $S_1M_3$  (*Cassia fistula* grown in soil + cocopeat media) whereas, the maximum days (15.50) taken for germination was in the interaction treatment of  $S_3M_5$ . In  $D_2$  sowing (15<sup>th</sup> August), similar results were obtained in sowing of 15<sup>th</sup> July with maximum (16.78) and minimum (9.18) days to germination (Table 1). In  $D_3$  sowing (15<sup>th</sup> September), earlier germination (11.25 days) was obtained in the treatment  $S_1M_3$  whereas, the maximum days (18.46) were taken to germinate the seeds in seed ball were in the interaction treatment of  $S_3M_1$ . The enhanced and early germination observed in media containing cocopeat can be attributed to its higher water holding capacity (WHC) and increased porosity, facilitating greater water availability to seed sown. The augmented water absorption contributes to higher germination percentage and accelerating the overall process. Additionally, the conducive climate during monsoon rains (23.2 cm in July) creates favorable conditions for seed germination, eliminating potential water stress (Fig. 1). Seedballs sowing followed by 15mm rain enhanced the germination in pearl millet as seed ball enhances nutrient



**Fig. 1.** Pooled average temperature (°C), relative humidity (%) and rainfall (mm) during the experiment

enrichment around the seed (Muhlana 2013). The coated seeds exhibit higher germination percentages in both laboratory and field conditions, safeguarding them from predators and reducing dormancy respectively (Choi et al., 2008; Gornish 2019; Jin et al., 2023). Cocopeat-containing seedballs yielded higher germination percentages in *Stereospermum suaveolens* (Trivedi and Joshi 2014). Similarly, *Bryonia laciniosa*, *Datura stramonium*, *Pongamia pinata* exhibited 100 per cent germination in seedball composed of soil: cow dung (1:1) and soil: cocopeat (1:1) (Patil et al., 2022). These results align with the outcomes of Afzal et al. (2020), emphasizing the advantages of coated seeds for achieving earlier germination.

**Growth parameters:** Plant height was significantly affected by the treatments in this study (Table 2). In sowing of 15<sup>th</sup> July (D<sub>1</sub>), the maximum (39.51 cm) and the minimum (21.89 cm) plant heights were exhibited by S<sub>1</sub>M<sub>3</sub> (*Cassia fistula* grown in soil + cocopeat media) and S<sub>3</sub>M<sub>2</sub> (*Schleichera oleosa* in soil + FYM media) treatments, respectively. Similar trend as in 15<sup>th</sup> July sowing period was observed of plant height under sowing of 15<sup>th</sup> August and 15<sup>th</sup> September. In sowing of 15<sup>th</sup> July, S<sub>3</sub>M<sub>4</sub> (*Putranjiva roxburghii* in soil + cocopeat + clay media) and S<sub>2</sub>M<sub>2</sub> (*Azadirachta indica* in soil + FYM media) resulted the maximum (15.88) and the minimum (5.89) leaf number, respectively (Table 2). In 15<sup>th</sup> August sowing, *Putranjiva roxburghii* seeds grown in soil + cocopeat media and *Azadirachta indica* in soil + FYM media exhibited the maximum (18.43) and the minimum (6.44) number of leaves,

respectively. In 15<sup>th</sup> September sowing, similar trend as in sowing of 15<sup>th</sup> August was observed regarding number of leaves. In case of 15<sup>th</sup> July sowing, the maximum (73.11 cm<sup>2</sup>) and minimum (18.65 cm<sup>2</sup>) leaf areas were obtained in S<sub>1</sub>M<sub>3</sub> (*Cassia fistula* in soil + cocopeat media) and S<sub>3</sub>M<sub>2</sub> (*Putranjiva roxburghii* in soil + FYM media), respectively (Table 3). Similar trend of leaf area was observed in sowing of 15<sup>th</sup> August and 15<sup>th</sup> September as in 15<sup>th</sup> July sowing.

Different media of seed balls significantly affected the stem girth of tree species (Table 3). In sowing of 15<sup>th</sup> July (D<sub>1</sub>), the maximum (26.22 mm) and the minimum (17.01 mm) stem girth were exhibited by S<sub>2</sub>M<sub>3</sub> (*Azadirachta indica* in soil + cocopeat media) and S<sub>3</sub>M<sub>2</sub> (*Putranjiva roxburghii* in soil + FYM media), respectively. In D<sub>2</sub> sowing (15<sup>th</sup> August), the maximum (29.01 mm) and the minimum (18.72 mm) stem girth were obtained in S<sub>2</sub>M<sub>3</sub> (*Azadirachta indica* in soil + cocopeat media) and S<sub>4</sub>M<sub>2</sub> (*Pongamia pinnata* in soil + FYM media), respectively. In sowing of D<sub>3</sub> (15<sup>th</sup> September), similar trend of stem girth as in sowing of 15<sup>th</sup> July was obtained. *Azadirachta indica* germinated and established well in cocopeat used media which further enhanced its growth. Therefore, the stem girth was more in this than other species.

The growth attributes of the plants showed superior results when planted on August 15<sup>th</sup>, possibly influenced by the favorable characteristics of the planting media, which facilitated enhanced germination and establishment of the seedballs. The climatic conditions during this period, marked by frequent monsoon rains in Punjab, provided an optimal

**Table 1.** Effect of different media on the germination percentage (%) and number of days to germination of trees species

	Germination percentage (%)																	
	D <sub>1</sub> (15 <sup>th</sup> July)						D <sub>2</sub> (15 <sup>th</sup> August)						D <sub>3</sub> (15 <sup>th</sup> September)					
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean
M <sub>1</sub>	75.00	76.00	52.33	70.00	70.67	68.80 <sup>d</sup>	78.00	73.67	49.67	65.00	70.33	67.33 <sup>d</sup>	63.00	63.67	42.00	59.00	60.00	57.53 <sup>c</sup>
M <sub>2</sub>	78.67	81.33	52.00	76.67	79.33	73.60 <sup>bc</sup>	78.67	80.33	53.00	71.00	72.00	71.00 <sup>bc</sup>	69.00	65.67	45.00	64.00	63.33	61.40 <sup>b</sup>
M <sub>3</sub>	89.00	90.00	60.67	79.00	80.00	79.73 <sup>a</sup>	88.33	89.67	57.67	75.33	80.00	78.20 <sup>a</sup>	75.67	78.00	50.00	70.67	71.00	69.07 <sup>a</sup>
M <sub>4</sub>	85.33	87.67	57.67	75.33	75.67	76.33 <sup>b</sup>	83.00	85.67	55.33	72.67	72.00	73.73 <sup>b</sup>	71.67	73.33	48.33	72.67	67.33	66.67 <sup>a</sup>
M <sub>5</sub>	80.00	84.67	53.33	74.33	71.00	72.67 <sup>c</sup>	80.33	80.00	52.00	70.67	70.00	70.60 <sup>c</sup>	68.00	70.00	45.00	66.67	65.00	62.93 <sup>b</sup>
Mean	81.60 <sup>a</sup>	83.93 <sup>a</sup>	55.20 <sup>c</sup>	75.07 <sup>b</sup>	75.33 <sup>b</sup>		81.67 <sup>a</sup>	81.87 <sup>a</sup>	53.53 <sup>c</sup>	70.93 <sup>b</sup>	72.87 <sup>b</sup>		69.47 <sup>a</sup>	70.13 <sup>a</sup>	46.07 <sup>c</sup>	66.60 <sup>b</sup>	65.33 <sup>b</sup>	
	Number of days to germination (Days)																	
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean
M <sub>1</sub>	8.21	9.15	15.00	12.00	15.05	11.88 <sup>ab</sup>	11.25	13.00	16.17	12.00	15.67	13.62 <sup>a</sup>	13.50	12.67	18.46	17.54	12.97	15.03 <sup>a</sup>
M <sub>2</sub>	8.00	8.85	14.89	10.76	14.56	11.41 <sup>b</sup>	10.05	10.00	16.00	10.45	14.00	12.10 <sup>b</sup>	12.15	11.50	17.73	16.00	13.00	14.08 <sup>b</sup>
M <sub>3</sub>	7.35	7.50	13.00	9.95	13.25	10.21 <sup>c</sup>	9.18	9.45	15.23	9.50	13.00	11.27 <sup>c</sup>	11.25	11.45	17.02	15.00	11.45	13.23 <sup>c</sup>
M <sub>4</sub>	7.67	8.25	13.00	10.00	13.45	10.47 <sup>c</sup>	9.75	9.78	15.83	10.00	13.76	11.82 <sup>bc</sup>	12.00	12.00	17.00	16.56	12.32	13.98 <sup>b</sup>
M <sub>5</sub>	9.00	10.00	15.50	11.13	15.00	12.13 <sup>a</sup>	11.56	12.00	16.78	12.34	14.02	13.34 <sup>a</sup>	14.00	13.00	18.34	17.89	13.56	15.36 <sup>a</sup>
Mean	8.05 <sup>d</sup>	8.75 <sup>c</sup>	14.28 <sup>a</sup>	10.77 <sup>b</sup>	14.26 <sup>a</sup>		10.36 <sup>c</sup>	10.85 <sup>c</sup>	16.00 <sup>a</sup>	10.86 <sup>c</sup>	14.09 <sup>b</sup>		12.58 <sup>c</sup>	12.12 <sup>c</sup>	17.71 <sup>a</sup>	16.60 <sup>b</sup>	12.66 <sup>c</sup>	

The mean followed by different alphabetical letters were computed using Duncan's Multiple Range Test are significantly different (p<0.05)

environment for plant growth (Fig. 1), contributing to the observed improvements in growth on August 15<sup>th</sup>. The frequent rains and bright sunlight initiate the new growth and better growth of the existing leaves and help the plant in maintaining the proper physiological functions. The obtained

results of growth attributes were better in the planting of 15<sup>th</sup> August. The results of plant height align with Bhardwaj (2014) who emphasized the positive impact of cocopeat media on seedling growth. Increased nutrient availability has been linked to improved shoot growth and yield in sorghum

**Table 2.** Effect of different media on the plant height (cm) and number of leaves after 9 months of sowing of trees species

	Plant height (cm)																	
	D <sub>1</sub> (15 <sup>th</sup> July)						D <sub>2</sub> (15 <sup>th</sup> August)						D <sub>3</sub> (15 <sup>th</sup> September)					
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean
M <sub>1</sub>	34.52	31.89	22.45	29.66	30.51	29.81 <sup>c</sup>	35.71	35.61	24.81	32.67	31.67	32.09 <sup>c</sup>	32.12	31.65	19.87	29.41	25.12	27.63 <sup>c</sup>
M <sub>2</sub>	32.89	30.05	21.89	28.92	28.69	28.49 <sup>c</sup>	34.09	33.52	23.77	31.55	30.50	30.69 <sup>c</sup>	30.09	29.75	19.03	27.99	23.89	26.15 <sup>c</sup>
M <sub>3</sub>	39.51	37.77	25.54	34.61	33.61	34.21 <sup>a</sup>	40.35	40.31	29.51	37.21	35.71	36.62 <sup>a</sup>	38.12	35.98	23.15	32.78	30.88	32.18 <sup>a</sup>
M <sub>4</sub>	37.65	35.33	24.67	32.85	32.55	32.61 <sup>b</sup>	39.11	36.88	27.66	36.41	33.78	34.77 <sup>b</sup>	36.87	34.21	22.61	32.02	28.76	30.89 <sup>a</sup>
M <sub>5</sub>	36.41	33.78	23.71	31.15	31.98	31.41 <sup>b</sup>	38.81	37.89	26.01	34.87	32.18	33.95 <sup>b</sup>	35.44	32.87	20.18	30.95	26.20	29.13 <sup>b</sup>
Mean	36.20 <sup>a</sup>	33.76 <sup>b</sup>	23.65 <sup>d</sup>	31.44 <sup>c</sup>	31.47 <sup>c</sup>		37.61 <sup>a</sup>	36.84 <sup>a</sup>	26.35 <sup>d</sup>	34.54 <sup>b</sup>	32.77 <sup>c</sup>		34.53 <sup>a</sup>	32.89 <sup>b</sup>	20.97 <sup>e</sup>	30.63 <sup>c</sup>	26.97 <sup>d</sup>	
	Number of leaves																	
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean
M <sub>1</sub>	8.42	6.21	7.46	11.62	13.78	9.50 <sup>c</sup>	9.33	7.01	8.40	14.89	16.03	11.13 <sup>d</sup>	7.38	6.01	6.98	13.51	14.31	9.64 <sup>c</sup>
M <sub>2</sub>	7.56	5.89	7.10	10.98	12.89	8.88 <sup>d</sup>	8.78	6.44	8.00	14.21	15.61	10.61 <sup>e</sup>	7.01	5.88	6.41	13.16	13.59	9.21 <sup>c</sup>
M <sub>3</sub>	10.31	8.01	8.45	13.65	15.67	11.22 <sup>a</sup>	11.31	9.55	9.20	16.98	18.43	13.09 <sup>a</sup>	9.67	7.66	8.55	14.58	16.41	11.37 <sup>a</sup>
M <sub>4</sub>	9.78	7.34	8.17	13.55	15.88	10.94 <sup>a</sup>	10.77	8.21	8.98	16.22	17.39	12.31 <sup>b</sup>	8.51	7.12	8.05	14.02	15.79	10.70 <sup>b</sup>
M <sub>5</sub>	9.21	6.87	7.67	12.04	14.34	10.03 <sup>b</sup>	10.43	7.65	8.72	15.31	16.98	11.82 <sup>c</sup>	8.11	6.52	7.65	13.86	15.25	10.28 <sup>b</sup>
Mean	9.06 <sup>c</sup>	6.86 <sup>e</sup>	7.77 <sup>d</sup>	12.37 <sup>b</sup>	14.51 <sup>a</sup>		10.12 <sup>c</sup>	7.77 <sup>e</sup>	8.66 <sup>d</sup>	15.52 <sup>b</sup>	16.89 <sup>a</sup>		8.14 <sup>c</sup>	6.64 <sup>e</sup>	7.53 <sup>d</sup>	13.83 <sup>b</sup>	15.07 <sup>a</sup>	

The mean followed by different alphabetical letters were computed using Duncan's Multiple Range Test are significantly different (p≤0.05)

**Table 3.** Effect of different media on the leaf area (cm<sup>2</sup> ground area) and stem girth (mm) of trees species

	Leaf area (cm <sup>2</sup> ground area)																	
	D <sub>1</sub> (15 <sup>th</sup> July)						D <sub>2</sub> (15 <sup>th</sup> August)						D <sub>3</sub> (15 <sup>th</sup> September)					
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean
M <sub>1</sub>	69.18	26.65	57.99	21.87	19.02	38.94 <sup>c</sup>	70.73	28.15	61.89	23.87	20.17	40.96 <sup>cd</sup>	63.76	24.08	56.25	21.37	19.89	37.07
M <sub>2</sub>	67.89	25.91	56.78	21.05	18.65	38.06 <sup>c</sup>	68.87	28.01	60.25	22.69	19.34	39.83 <sup>d</sup>	62.56	23.79	55.46	21.01	19.02	36.37 <sup>d</sup>
M <sub>3</sub>	73.11	29.18	62.17	25.88	23.17	42.70 <sup>a</sup>	75.28	32.11	67.39	27.65	24.75	45.44 <sup>a</sup>	71.67	28.66	60.59	24.79	23.15	41.77 <sup>a</sup>
M <sub>4</sub>	72.98	28.75	60.88	24.75	21.66	41.81 <sup>ab</sup>	74.01	30.87	64.22	26.71	22.17	43.60 <sup>ab</sup>	68.98	26.16	59.13	23.45	21.66	39.88 <sup>ab</sup>
M <sub>5</sub>	70.54	28.09	59.18	22.10	19.86	39.96 <sup>bc</sup>	72.87	29.77	63.91	25.01	20.98	42.51	66.15	25.84	57.89	22.71	20.51	38.62
Mean	70.74 <sup>a</sup>	27.72 <sup>c</sup>	59.40 <sup>b</sup>	23.13 <sup>d</sup>	20.47 <sup>e</sup>		72.35 <sup>a</sup>	29.78 <sup>c</sup>	63.53 <sup>b</sup>	25.19 <sup>d</sup>	21.48 <sup>e</sup>		66.62 <sup>a</sup>	25.71 <sup>c</sup>	57.86 <sup>b</sup>	22.67 <sup>d</sup>	20.85 <sup>d</sup>	
	Stem girth (mm)																	
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean
M <sub>1</sub>	20.56	21.29	18.76	17.75	17.81	19.23 <sup>d</sup>	23.56	24.68	21.88	19.17	20.08	21.87 <sup>d</sup>	19.92	18.01	18.25	17.97	17.67	18.37 <sup>d</sup>
M <sub>2</sub>	19.01	20.98	17.97	17.18	17.01	18.43 <sup>d</sup>	21.89	23.12	20.02	18.72	19.55	20.66 <sup>e</sup>	18.25	17.48	17.82	17.45	16.99	17.60 <sup>d</sup>
M <sub>3</sub>	24.56	26.22	23.11	21.12	20.88	23.18 <sup>a</sup>	28.88	29.01	25.03	22.89	23.92	25.95 <sup>a</sup>	23.78	23.79	21.95	19.90	20.52	21.99 <sup>a</sup>
M <sub>4</sub>	23.07	24.89	21.79	20.88	19.01	21.93 <sup>b</sup>	26.03	27.81	23.12	21.61	22.08	24.13 <sup>b</sup>	22.91	21.53	20.37	19.07	19.02	20.58 <sup>b</sup>
M <sub>5</sub>	21.89	23.04	20.56	18.12	18.68	20.46 <sup>c</sup>	25.19	26.04	22.35	20.26	21.41	23.05 <sup>c</sup>	21.28	19.99	19.27	18.75	18.78	19.62 <sup>c</sup>
Mean	21.82 <sup>b</sup>	23.28 <sup>a</sup>	20.44 <sup>c</sup>	19.01 <sup>d</sup>	18.68 <sup>d</sup>		25.11 <sup>b</sup>	26.13 <sup>a</sup>	22.48 <sup>c</sup>	20.53 <sup>d</sup>	21.41 <sup>d</sup>		21.23 <sup>a</sup>	20.16 <sup>b</sup>	19.53 <sup>bc</sup>	18.63 <sup>c</sup>	18.60 <sup>c</sup>	

The mean followed by different alphabetical letters were computed using Duncan's Multiple Range Test are significantly different (p≤0.05)

**Table 4.** Effect of different media on the survival percentage (%) of sowing of trees species

	D <sub>1</sub> (15 <sup>th</sup> July)						D <sub>2</sub> (15 <sup>th</sup> August)						D <sub>3</sub> (15 <sup>th</sup> September)					
	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	Mean
M <sub>1</sub>	47.00	39.00	34.00	41.00	39.00	40.00 <sup>bc</sup>	46.67	45.00	37.67	43.00	44.00	43.27 <sup>b</sup>	41.33	35.00	34.00	34.33	35.00	35.93 <sup>b</sup>
M <sub>2</sub>	46.00	40.00	34.00	37.00	41.33	39.67 <sup>c</sup>	45.00	44.00	36.00	43.00	45.00	42.60 <sup>b</sup>	41.00	35.00	33.00	34.00	38.00	36.20 <sup>b</sup>
M <sub>3</sub>	47.33	45.33	37.00	44.00	45.67	43.87 <sup>a</sup>	50.67	50.00	40.67	46.67	47.00	47.00 <sup>a</sup>	41.00	41.00	34.00	37.67	39.00	38.53 <sup>a</sup>
M <sub>4</sub>	45.00	42.00	39.67	41.00	40.00	41.53 <sup>bc</sup>	49.00	49.33	39.00	44.00	47.33	45.73 <sup>a</sup>	41.00	37.00	35.00	37.00	34.00	36.80 <sup>b</sup>
M <sub>5</sub>	45.00	43.00	35.67	43.33	41.00	41.60 <sup>b</sup>	47.00	46.33	36.00	41.56	46.00	43.38 <sup>b</sup>	40.00	38.67	31.00	38.00	35.00	36.53 <sup>b</sup>
Mean	46.07 <sup>a</sup>	41.87 <sup>b</sup>	36.07 <sup>c</sup>	41.27 <sup>b</sup>	41.40 <sup>b</sup>		47.67 <sup>a</sup>	46.93 <sup>ab</sup>	37.87 <sup>d</sup>	43.65 <sup>c</sup>	45.87 <sup>b</sup>		40.87 <sup>a</sup>	37.33 <sup>b</sup>	33.40 <sup>c</sup>	36.20 <sup>t</sup>	36.20 <sup>b</sup>	

The mean followed by different alphabetical letters were computed using Duncan's Multiple Range Test are significantly different ( $p \leq 0.05$ )

**Table 5.** pH and EC (dS/m) of different media treatments

Treatments	pH	EC (dS/m)
M <sub>1</sub> [Soil + Clay (1:1 v/v)]	7.60 <sup>b</sup>	2.80 <sup>b</sup>
M <sub>2</sub> [Soil + FYM (1:1 v/v)]	7.80 <sup>a</sup>	0.25 <sup>e</sup>
M <sub>3</sub> [Soil + Cocopeat (1:1 v/v)]	6.80 <sup>d</sup>	0.85 <sup>d</sup>
M <sub>4</sub> [Soil + Cocopeat + Clay (4:3:3)]	6.50 <sup>e</sup>	2.10 <sup>c</sup>
M <sub>5</sub> (Seed priming with clay)	7.40 <sup>c</sup>	3.40 <sup>a</sup>

(Tamilarasan et al., 2021b). The use of clay in seed balls demonstrated enhanced seedling establishment even in the presence of predators (Overdyck et al., 2013). Plants exhibiting robust growth displayed a higher number of leaves (Sudrajat and Rustam 2020). However, the specific number of leaves varied based on the growth habits and the physiology of individual tree species in coated seeds of drumstick tree (Soares et al., 2023). Environmental factors such as light, temperature, and nutrient availability, along with overall plant health, influenced vegetative attributed as leaf area, stem girth, maximum fresh and dry weight of the leaves. Well-established seedlings originating from seedballs typically develop robust root and shoot systems, enabling efficient nutrient and water uptake from the soil and ensuring effective photosynthesis. This nutritional enrichment contributes to enhanced plant growth and vigor. Further, growth was also improved when seed balls were broadcasted on the uncultivated land followed by monsoon (Maity et al., 2015). Gawankar et al. (2019) also observed that cocopeat media increased the seedling vigour which may contribute to the increase in fresh and dry weight.

**Field survival percentage:** In sowing of 15<sup>th</sup> July (D<sub>1</sub>), the maximum (47.33 %) survival percentage was exhibited by S<sub>1</sub>M<sub>3</sub> (*Cassia fistula* grown in soil + cocopeat media) whereas, the minimum (34.00 %) survival percentage was obtained in S<sub>3</sub>M<sub>1</sub> (*Schleichera oleosa* in soil + clay media) and S<sub>3</sub>M<sub>2</sub> (*Schleichera oleosa* in soil + FYM media) (Table 4). In D<sub>2</sub> (15<sup>th</sup> August) sowing, S<sub>1</sub>M<sub>3</sub> exhibited the maximum (50.67 %) survival percentage and minimum (36.00 %) was in S<sub>3</sub>M<sub>2</sub> and S<sub>3</sub>M<sub>5</sub> (*Schleichera oleosa* grown in seed priming with clay). In

sowing time of 15<sup>th</sup> September (D<sub>3</sub>), again the maximum (41.33 %) survival percentage was exhibited by *Cassia fistula* in soil + cocopeat media), whereas, minimum (31.00 %) survival percentage was obtained in *Schleichera oleosa* in seed priming with clay. The germinated seedlings received frequent rains by monsoon which provided favorable climate for their growth and survival. Loss due to predatory animals and bird also results in higher survival rate as observed in paddy (Fenangad and Orge 2015). Improved establishment and survival of *Leucaena leucocephala* using seed ball technique due to low abiotic stress and higher nutrient availability near rhizosphere (Tamilarasan et al., 2021a). Moreover, the seedball technique reduce the pathogen infestation which results in healthy seedlings which further influence the survival rate and establishment as concluded by Afzal et al. (2020). Sudrajat and Rustam (2020) and Qiu et al. (2020) also concluded that the coated seeds exhibited better establishment of the growing seedlings, hence more survival rate.

Media M<sub>3</sub> (Soil + cocopeat) had the closest pH (6.80) to the neutral pH (7.0). The pH of the used media was be in order M<sub>2</sub>>M<sub>1</sub>>M<sub>5</sub>>M<sub>3</sub>>M<sub>4</sub> (7.80 > 7.60 > 7.40 > 6.80 > 6.50). In all the above-mentioned results of all parameters, M<sub>3</sub> (soil + cocopeat) exhibited best results (Table 4). In case of EC, the media M<sub>5</sub> (seed priming with clay) exhibited maximum EC (3.40 dS/m) whereas, M<sub>2</sub> (soil + FYM) exhibited minimum EC (0.25 dS/m). EC of all used media was in order of M<sub>5</sub>>M<sub>1</sub>>M<sub>4</sub>>M<sub>3</sub>>M<sub>2</sub>.

## CONCLUSION

Seedball technique can be promising to produce the quality tree seedling on unmanaged and degraded landscape where the seedling survival is major concern. Through the meticulous examination of five avenue tree species and five growing media mixtures of seed ball, it becomes evident that the choice of media for enclosing seedballs significantly influences the germination and subsequent establishment of seeds in natural conditions and

followed by better survival success. The combination of soil and cocopeat emerged as the most conducive growing medium for preparing the seed balls for successful germination and robust plant development, particularly when sown in August. *Azadirachta indica* and *Cassia fistula* hold great potential for the broader application of seed ball technology in environmental restoration efforts for avenue tree plantations.

#### AUTHORS' CONTRIBUTIONS

R K Dubey, Madhu Bala and Simrat Singh developed the concept of the experiment. Arshdeep Singh executed the trial and collected and analysed the data. Arshdeep Singh, R K Dubey, Arshdeep Kaur and Kritika Pant prepared the manuscript.

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# Effect of Seed Priming with GA<sub>3</sub> on Quality Attributes of Harvested Seeds of Sunflower under Mid-Himalayan Region of Himachal Pradesh

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**Abstract:** To investigate the effect of seed priming with GA<sub>3</sub> on enhancement of seed quality attributes in sunflower, analysis of harvested seeds was done at Department of Seed Science and Technology, Dr YS Parmar UHF, Nauni, Solan during the year 2021-22. Under field conditions, there were ten different seed priming treatments i.e. 8 concentrations of GA<sub>3</sub> (25, 50, 75, 100, 125, 150, 175, 200 ppm) and hydropriming, both for a duration of 12 hours along with control. Among all treatments GA<sub>3</sub> @ 100 ppm significantly enhanced the seed germination (92.25 %), speed of germination (54.66), seedling length (27.84 cm), seedling fresh weight (581.70 mg), seedling dry weight (28.71 mg), SVI-I (2567.79), SVI-II (2648.73), oil content (43.81 %) and resulted in the lowest EC (322.75 μS/m) of harvested seeds. Therefore, seed primed with GA<sub>3</sub> @ 100 ppm for 12 hours could be recommended for improving the seed quality attributes in sunflower.

**Keywords:** DRS-1, GA<sub>3</sub>, Oil content, Seed priming, Sunflower, *Helianthus annuus* L.

Sunflower (*Helianthus annuus* L.), a member of the Asteraceae family, is a highly sought-after oil-yielding plant in the Indian subcontinent (Das et al 2019). Holds a prominent position as one of the world's top three productive oilseed crops (Ahmed et al., 2020). In India, during the 2019-20 season, sunflower cultivation spanned an area of 0.24 million hectares, resulting in a production of 0.22 million tonnes and productivity rate of 891 kg/ha (Anonymous 2020). Despite their many benefits, sunflowers are becoming less prevalent in agricultural areas for a number of reasons, such as being unable to harvest their full potential along food value chains due to unfavorable weather and a shortage of viable seeds for farmers (Bassegio et al., 2016). Therefore, in order to minimize the problems, some seed quality enhancement treatments like priming need to be employed. Choudhary et al (2008) observed that seed priming is a controlled hydration process that restricts germination while facilitating essential physiological and biochemical changes. Among the various priming agents, gibberellic acid (GA<sub>3</sub>) is widely recognized and utilized due to its pivotal role in growth and development processes and effectively breaks seed dormancy, promotes germination, stimulates internodal elongation, facilitates hypocotyl growth and induces cell division in the cambial zone (Yamaguchi 2008). Furthermore, demonstrated tolerance to adverse abiotic conditions and exhibit an earlier onset of blooming and maturation, contributing to their overall resilience and productivity (Ulfat et al., 2017). The present investigation aimed to study the effect of seed priming with

GA<sub>3</sub> to enhance quality attributes of harvested seeds in sunflower grown under mid-Himalayan region of H.P.

## MATERIAL AND METHODS

The study was conducted at Department of Seed Science and Technology, Dr YS Parmar UHF, Nauni, Solan (H.P.) during the year 2021-22. The seeds of sunflower var. 'DRSH-1' from the field trial were harvested and evaluated for various parameters. In field trial, there were ten different seed priming treatments all for a duration of 12 hours (Table 1). Observations on different parameters including seed germination (%), speed of germination, seedling length (cm), seedling fresh weight (mg), seedling dry weight (mg), seed vigour indices, electrical conductivity (μS/m) and oil content (%) were performed as per the standard procedures.

For computation of seed germination, the seeds were allowed to germinate using paper towel method at 25°C in the seed germinator.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds used}} \times 100$$

Speed of germination was recorded by counting the number of seedlings emerged each day up to final count of seedling emergence and rate of germination (Maguire 1962).

Speed of germination =

$$\frac{\text{Number of normal seedlings}}{\text{Days to first count}} + \dots + \frac{\text{Number of normal seedlings}}{\text{Days to final count}}$$

For measuring the seedling length, ten normal seedlings



were selected at random from each treatment combination. The seedling length was worked out by measuring the total length of each seedling from the tip of the apex leaf to the tip of primary root with the help of a scale and expressed as mean value in centimetre (cm). For calculation of seedling fresh weight, ten normal seedlings selected for seedling length were weighed on an electronic balance and average was worked out and expressed in milligrams (mg). For seedling dry weight ten seedlings were wrapped in butter paper pockets and kept in oven at 50°C for 48 hours. Then, dry weight of seedlings was recorded and the mean value was expressed in milligrams (mg). Seed vigour index-I and II was calculated (Abdul-Baki and Anderson 1973)

Seed vigour index-I = Germination percentage (%) × Seedling length (cm)

Seed vigour index-II = Germination percentage (%) × Seedling dry weight (mg)

For electrical conductivity, four replications of 0.5 g seeds of each treatment were weighed and soaked in 100 ml distilled water and incubated for 24 hours at 25 °C in the dark. At first, the electrical conductivity of distilled water was measured and then the electrical conductivity of the leachate was measured using electrical conductivity meter and actual value was worked out.

Actual electrical conductivity of seeds = Electrical conductivity of leachate - Electrical conductivity of distilled water

Oil content was calculated using Soxhlet Extraction apparatus

$$\% \text{ crude fat} = (W_2 - W_1) \times \frac{100}{S}$$

Where,  $W_1$  = Weight of empty flask (g),  $W_2$  = Weight of flask and extracted fat (g),  $S$  = Weight of sample

**Statistical analysis:** This was done by with windows-based computer application OPSTAT (Sheoran 2006).

## RESULTS AND DISCUSSION

**Seed germination (%):** The various priming treatments on sunflower seeds var. DRSH-1 significantly affected seed germination (Table 1). The seeds primed with  $GA_3$  @ 100 ppm for 12 hours resulted in maximum seed germination (92.25%), while the minimum seed germination (74.75%) was in un-primed seeds. This might be due to accelerated biochemical, metabolic and molecular processes that resulted from the priming of the seeds and led to increased accumulation of enzymatic, nuclear and cytoplasmic contents in the cells thereby leading to establishment of good crop stand in the field (Baskin and Baskin 2014).

**Speed of germination:** The seeds primed with  $GA_3$  @ 100 ppm for 12 hours resulted in maximum speed of germination

(54.66) and minimum (34.90) in un-primed seeds. The priming with  $GA_3$  has resulted in bolder seeds with thicker seed coat and higher weight that has ensured quick germination. In addition to this, these seeds had larger concentrations of carbohydrates, proteins and RNA, which accelerates the biochemical and metabolic processes involved in germination and led to faster rate of germination (Pavitrarnata et al., 2023). Kumar and Singh (2013) also observed similar results in bitter gourd seeds.

**Seedling length (cm):** The seeds that were primed with  $GA_3$  @ 100 ppm for 12 hours resulted in maximum seedling length (27.84 cm) and minimum (20.50 cm) in un-primed seeds. The longer seedlings in seeds primed with  $GA_3$  @ 100 ppm may be due to the hastening of numerous processes and reactions during seed germination which led to the improved crop stand establishment in the field (Pallaoro et al., 2016). The results are very similar to Selvakumari et al. (2007) in sweet william and Zahedi et al (2012) in china aster.

**Seedling fresh weight (mg):** The seeds that were primed with  $GA_3$  @ 100 ppm for 12 hours gave highest fresh weight (581.70 mg) and untreated seeds had the lowest fresh weight (441.48 mg). The increased seedling fresh weight after seed priming with  $GA_3$  @ 100 ppm due to enhance water uptake of seedling which may have activated enzymes and mobilized the reserve materials that were transported in the embryo resulting stronger seedlings as a result of improved growth of embryo. Similar findings were reported by Eisvand et al. (2011) in carrot.

**Seedling dry weight (mg):** The seeds primed with  $GA_3$  @ 100 ppm for 12 hours exhibited maximum seedling dry weight (28.71 mg), while minimum seedling dry weight (26.27 mg) was found in un-primed seeds. Pratibha et al. (2015) also reported the similar results in papaya.

**Seed vigour indices:** The maximum value of seed vigour



Plate 1. Seed germination (%) of harvested seed  $T_1$  and  $T_6$

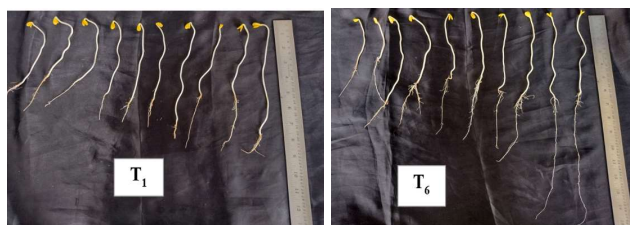


Plate 2. Seedling length (cm) of harvested seed in treatments  $T_1$  and  $T_6$

index-I (2567.79) was in seeds that were primed with GA<sub>3</sub> @ 100 ppm for 12 hours and minimum (1531.89) was in control. The seeds primed with GA<sub>3</sub> @ 100 ppm had the highest value for seed vigour index-I, which may be due to the reason that these seeds had demonstrated the highest percentage of germination and resulted in the seedlings that were tall and robust. Seeds with higher germination percentage and taller seedlings have contributed to the higher seed vigour index-I. Similar results were found by Yari et al. (2011) in bell pepper.

Similarly, seed vigour index-II was maximum (2648.73) in seeds that were primed with GA<sub>3</sub> @ 100 ppm for 12 hours and was found to be minimum (1888.04) in control. The higher seedling vigour of the seeds harvested from the plants obtained from the GA<sub>3</sub> (100 ppm) primed seeds resulted in

the production of much bolder and better quality seeds in the plants. GA<sub>3</sub> primed seeds displayed higher percentage of germination, increased seedling length and seedling dry weight too and this accounted for higher seed vigour index-II. These results are in confirmation with the results of Arefi et al. (2012) in caper seeds.

**Electrical conductivity ( $\mu\text{S/m}$ ):** The seeds that were primed with GA<sub>3</sub> @ 100 ppm exhibited minimum seed EC (322.75  $\mu\text{S/m}$ ) whereas, the maximum (466.50  $\mu\text{S/m}$ ) was in unprimed seeds. Electrical conductivity of seeds was reported maximum in control and may be due to the facts that the seeds might have leaked more solutes when hydrated in water and hence became less vigorous. The minimum electrical conductivity with the GA<sub>3</sub> (100 ppm) treated seeds

**Table 1.** Effect of GA<sub>3</sub> seed priming for 12 hours on seed quality attributes of freshly harvested seeds in sunflower

Treatments	Seed germination (%) *	Speed of germination	Seedling length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
T <sub>1</sub> (Control)	74.75 (8.70)	34.90	20.50	441.48	25.26
T <sub>2</sub> (Hydropriming of seeds)	81.50 (9.08)	37.25	22.46	461.32	25.50
T <sub>3</sub> (Seed priming with GA <sub>3</sub> @ 25 ppm)	84.00 (9.22)	39.93	23.79	542.70	25.76
T <sub>4</sub> (Seed priming with GA <sub>3</sub> @ 50 ppm)	86.00 (9.33)	42.71	24.79	543.48	25.82
T <sub>5</sub> (Seed priming with GA <sub>3</sub> @ 75 ppm)	88.25 (9.45)	46.33	25.12	555.34	25.98
T <sub>6</sub> (Seed priming with GA <sub>3</sub> @ 100 ppm)	92.25 (9.66)	54.66	27.84	581.70	28.71
T <sub>7</sub> (Seed priming with GA <sub>3</sub> @ 125 ppm)	90.00 (9.54)	50.07	25.21	566.86	26.88
T <sub>8</sub> (Seed priming with GA <sub>3</sub> @ 150 ppm)	89.50 (9.51)	48.42	24.58	565.35	26.21
T <sub>9</sub> (Seed priming with GA <sub>3</sub> @ 175 ppm)	87.50 (9.41)	46.16	24.12	553.01	25.83
T <sub>10</sub> (Seed priming with GA <sub>3</sub> @ 200 ppm)	85.25 (9.29)	46.05	23.90	547.02	25.79
Mean	85.90 (9.32)	44.65	24.23	535.83	26.17
CD (p=0.05)	(0.09)	2.13	0.49	13.16	0.23

\*Figures in the parenthesis represent square root transformed values

**Table 2.** Effect of GA<sub>3</sub> seed priming on seed vigour indices, Seed EC and oil content of freshly harvested seeds in sunflower

Treatments	Seed Vigour Index-I (Length)	Seed Vigour Index-II (Mass)	Seed EC ( $\mu\text{S/m}$ )	Oil content (%)
T <sub>1</sub>	1531.89	1888.04	466.50	41.81
T <sub>2</sub>	1830.22	2078.31	434.50	42.38
T <sub>3</sub>	1997.94	2164.01	396.25	42.71
T <sub>4</sub>	2132.12	2220.51	354.25	42.75
T <sub>5</sub>	2216.87	2292.32	334.25	43.06
T <sub>6</sub>	2567.79	2648.73	322.75	43.81
T <sub>7</sub>	2268.49	2418.76	329.75	43.19
T <sub>8</sub>	2198.99	2345.93	361.75	43.13
T <sub>9</sub>	2110.05	2260.08	376.25	42.75
T <sub>10</sub>	2037.38	2198.17	389.25	42.50
Mean	2089.17	2251.48	376.55	42.81
CD (p=0.05)	44.09	52.74	2.28	0.84

See Table 1 for details

could be due to the reason that the seeds were highly vigorous and had leached minimum quantum of solutes. Zahedi et al. (2012) in sweet william and Selvakumari et al. (2007) in China aster also observed same results.

**Oil content (%):** The seeds that were primed with GA<sub>3</sub> @ 100 ppm for 12 hours resulted in maximum oil content (43.81 %) which was statistically at par with T<sub>7</sub> (43.19 %), T<sub>8</sub> (43.12 %) and T<sub>5</sub> (43.06 %). The minimum oil content was in un-primed seeds (41.81 %).

### CONCLUSION

Seed priming with GA<sub>3</sub> @ 100 ppm for 12 hours was found superior among all the treatments tested for quality seed production and significantly improved all the seed quality attributes in sunflower. Therefore, can be recommended for producing quality seeds under mid-Himalayan region of Himachal Pradesh.

### AUTHORS CONTRIBUTION

BSD, designed the study and supervised the experiment; AT collected the data, performed the analysis and developed a draft of the manuscript; RS contributed in supervising and drafting; RV contributed in supervising and analysis; SK made contributions in the analysis and drafting; V assisted in data collection and drafting; and further, all the authors improved and approved the manuscript.

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# Household Level Assessment of Awareness of MSP and Source of Information Across States in India: Evidence from NSSO Data

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**Abstract:** The present study was planned to evaluate awareness of minimum support price (MSP) and households' preferred source of agricultural extension services for major cereals and coarse-cereals across the different states of India. Unit-level data of the Situation Assessment Survey (SAS) of Agricultural Households in India for 2018-19 was used for the two visits, viz. *Visit 1* (June-December, 2018) and *Visit 2* (January-July, 2019). Major cereals and coarse cereals, namely Paddy, Jowar, Bajra, Wheat, Maize, Ragi and Barley, were selected as these contribute more than 70 per cent of the total food grains production of India. Sample of 44,770 agricultural households for both visits was chosen to interpret the results better. The detailed population estimates were examined using the sample and corresponding weights across different states and households. More than 80 per cent of the agricultural households access technical advice from progressive farmers, input dealers and Radio/TV. For input-wise source of information is concerned, for improved seed/variety, agricultural households rely upon progressive farmers and agricultural universities, for fertilizer application input dealers and KVKs are the major sources of information, for plant protection measures the source of information was private commercial agents and Kisan Call Centres (KCC) while for harvesting and marketing, FPOs and Private processors are the major sources of information. The findings of the study would be helpful for both the public and private extension services to know the preferences of the agricultural households for information access for strengthening extension services in India.

**Keywords:** NSSO, MSP, Technical advice, Awareness, Sources of information

It is well recognized that agricultural extension contributes to a great deal in achieving food and nutritional security in India and improving farmers' profitability. The Indian agricultural extension system has recently undergone many changes, leading to amplified demand for agricultural extension systems (Ferroni and Zhou 2012). Although the share of agricultural extension as a share of agricultural gross domestic product (GDP) at the national level has improved since 1970, this slowed until 2004. Concurrently, only 0.54% of AgGross Domestic Product (AgGDP) was spent on research and extension during 2014-15 (Gulati et al., 2018), causing agrarian extension services have stagnated (Sajesh and Suresh, 2016). The T & V system of agricultural extension resulted in higher level of extension activity with faster knowledge diffusion in the 1980's. Currently, the extension services of India have been skewed towards the private extension system. The reason involves discouragement of the role and efficacy of the extension system in improving farm productivity and anticipation of the large-scale substitution of the public extension system coupled with the private extension system. On average, the available extension services reach only 6.8 percent of the farmers in the public extension system (Suresh et al., 2022). In Ethiopia fellow farmers were the most frequently used as source of agricultural information, with 36% of respondents

relying on them (Brhne et al., 2017). Sajesh and Suresh (2016) examined state-wise the number of operational holdings per extension personnel, which was the highest in Andhra Pradesh (3162), followed by Karnataka (2428) and found wide variation across the states. Gulati et al. (2018) observed that the hilly areas had one extension expert per 400 operational holdings; however, in irrigated areas, one extension expert served about 750 operational holdings.

In India, the majority ( $\approx 80\%$ ) of the farmers are small and marginal, and generally rely on local sources of information, i.e., progressive farmers and input dealers (Birthal et al., 2015). Usually, these sources of information have been questioned for their reliability, timings, and relevancy. These sources are consistently unable to give technical and suitable advice, as the country's agricultural system has diverse problems (Shah et al., 2021). There is dearth of extension personnel and poor linkages between these personnel and farmers, which exacerbates the challenges in disseminating agricultural information effectively. In the present scenario, farmers require a wide range of information to support their farm enterprises and access to timely, reliable and relevant information that can support the complexity within which their farm enterprises operate (Gupta and Shinde 2013). These arguments highlighted the demand for extension services to sustain farm productivity (Ferroni and Zhou 2012). Although

very few studies analyzed MSP awareness as a whole (Birthal et al., 2015 and Das 2020) and source-wise technical advice of the country, none focused on region-specific MSP awareness and source and types of information about the application of different inputs. The study would help to reorient public extension services with existing funds, as every region has their issues in agriculture in India. In this context, the present study was taken up to estimate the current status of awareness of MSP along with the source of information for technical advice across different states of India.

### MATERIAL AND METHODS

The unit-level data of the Situation Assessment Survey (SAS) of Agricultural Households in India conducted by the National Sample Survey Office (NSSO) for 2018-19 was used. The NSS survey has been conducted over two seasons, *Kharif* (Visit 1: June-December, 2018) and *Rabi* (Visit 2: January-July, 2019). For the present study, all the major cereals and coarse cereals, namely paddy, jowar, bajra, wheat, maize, ragi and barley, selected to observed farmers' awareness about MSP as well as the different sources of information about the input use. Furthermore, the states of Andhra Pradesh, Haryana, Madhya Pradesh, Maharashtra, Punjab, Uttar Pradesh, and West Bengal were selected as these states contribute more than 70 per cent of the total food grain production of India.

The weighted estimates of NSSO for every parameter were used after careful validation with published data of the NSSO report (SAS, 2018-19). The study included the common agricultural households during the visits (visit 1 and 2) of the survey, with the total number of agricultural households for India at 44,770. Detailed sample size and population estimate across all states can be referred from Table 1.

The summation of weights can estimate the general weighted count for categorical parameters,

$$\sum_{i=1}^n w_i \quad (1)$$

Similarly, the weighted sum for variables was estimated by,

$$\sum_{i=1}^n x_i w_i \quad (2)$$

And weighted mean was estimated as,

$$\frac{\sum_{i=1}^n x_i w_i}{\sum_{i=1}^n w_i} \quad (3)$$

Where,

x = variable of interest

w = weight assigned to each agricultural household in the study

i = agricultural households

### RESULTS AND DISCUSSION

#### Crop-wise and State-wise awareness of agricultural households about MSP:

Awareness of MSP of paddy stands at less than 50 percent during *Kharif* in all the selected states except Punjab and Madhya Pradesh. About 56 percent of the farmers in Madhya Pradesh and 52 percent in Punjab were aware of the paddy MSP. Undeniably, Government of India announced the MSP to the whole nation, but very few percent of the farmers from all the states were aware of the price policy. State-wise percentage of farmers' awareness of MSP of crops supports present argument. The states like Madhya Pradesh, Punjab, Haryana, and Rajasthan, where the major share of procurement is done by the procurement agencies, the awareness about the MSP is relatively higher than in the rest of the states. (Table 2). Similarly, more than 50 percent of bajra growers were aware of MSP in Punjab and Madhya Pradesh. The knowledge of MSP about maize crop about 57 per cent of farmers from Punjab and 37 per cent from Uttar Pradesh were aware of MSP for maize crop as the major proportion of maize procurement in the market yard is done by the private players and the traders.

Wheat is the principal crop, which has been grown across all the states except for South Indian states and knowledge about MSP of the wheat crop is more in regions like Punjab, Haryana and Madhya Pradesh. Only 17 percent of wheat farmers in West Bengal and 25 percent in Bihar were aware of the MSP. The farmers' awareness about MSP of wheat is more pronounced in Uttar Pradesh (37 per cent) and Rajasthan (35 percent). South Indian states like Tamil Nadu and Andhra Pradesh, paddy crop is also grown during *Rabi* season; about 62 and 49 percent of the farmers were aware of the MSP of paddy during *Rabi* season, respectively. Likewise, 53 per cent of farmers from West Bengal were aware of the MSP of paddy during rabi season.

#### State-wise source of agricultural extension services:

The major states, namely Tamil Nadu, Andhra Pradesh, and Haryana, are the topmost states which were able to provide technical advice regarding agricultural production during *Kharif* and *rabi* season (Fig. 1). However, in Punjab, about 39 percent of farmers in *Kharif* and 45 percent of farmers in *Rabi* are getting advice for agricultural production from different sources. Input dealers (range of 11.3 to 32.2 %) followed by Radio/TV/Newspaper (range of 2.6 to 26.5 %) are the most utilized sources of information for agricultural households across the state of Punjab. While almost all the states, progressive farmers were also an essential source of technical advice.

The veterinary department has emerged as the third most important information source for agricultural households in

**Table 1.** Crop and state wise sample of the agricultural households

States	Sample agricultural households										Estimated agricultural households										Estimated agricultura		
	Paddy	Jowar	Bajra	Maize	Ragi	Wheat	Barley	Paddy	Jowar	Bajra	Maize	Ragi	Wheat	Barley	Paddy	Jowar	Bajra	Maize	Ragi	Wheat		Barley	
<i>Kharif</i>																							
Andhra Pradesh	551	25	1	43	8	-	2	1032967	36493	3277	94663	8036	-	-	-	-	-	-	-	-	-	-	3130993
Bihar	2546	4	13	179	-	6	-	4203371	3179	39827	221187	-	7174	827	-	-	-	-	-	7174	827	-	7011314
Haryana	287	48	118	8	-	-	-	620365	69875	269957	612	-	-	-	-	-	-	-	-	-	-	-	1885059
Karnataka	269	190	53	255	203	15	7	536082	525104	181431	784893	352812	20980	23406	-	-	-	-	-	20980	23406	-	-
Madhya Pradesh	332	26	74	308	-	17	-	946539	106118	245164	833174	-	66040	-	-	-	-	-	-	66040	-	-	7127133
Punjab	657	7	2	27	-	-	-	823251	288	851	37055	-	-	-	-	-	-	-	-	-	-	-	1404923
Rajasthan	25	67	511	157	-	-	2	37751	267535	1393932	399719	-	-	4419	-	-	-	-	-	-	4419	-	7041525
Tamil Nadu	463	56	1	98	16	-	1	483923	116979	1996	130276	17767	-	170	-	-	-	-	-	-	170	-	-
Uttar Pradesh	1871	32	378	245	-	-	1	4101544	73818	1123058	750629	-	-	651	-	-	-	-	-	-	651	-	17588288
West Bengal	1730	1	1	48	-	-	-	3107966	7530	711	40831	-	-	-	-	-	-	-	-	-	-	-	6526557
<i>Rabi</i>																							
Andhra Pradesh	337	20	-	23	8	-	-	608439	45394	-	41957	6212	-	2246	-	-	-	-	-	-	2246	-	3130993
Bihar	48	-	-	687	9	2407	1	71022	-	-	-	-	-	32526	-	-	-	-	-	-	32526	-	7011314
Haryana	2	-	1	1	-	486	-	545	-	-	-	-	-	19713	-	-	-	-	-	-	19713	-	1885059
Karnataka	112	161	29	127	83	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Madhya Pradesh	-	-	2	18	-	1105	4	-	-	-	46728	-	3497116	7141	-	-	-	-	-	3497116	7141	-	7127133
Punjab	4	-	-	5	1	625	-	6542	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1404923
Rajasthan	8	2	6	5	36	658	31	549	894	2246	21428	126951	1769218	69173	-	-	-	-	-	1769218	69173	-	7041525
Tamil Nadu	455	39	6	43	8	1	-	539866	38903	32526	31655	7629	270	-	-	-	-	-	-	270	-	-	-
Uttar Pradesh	10	7	-	53	1	2507	9	25382	25304	19713	193280	286	5764480	17927	-	-	-	-	-	5764480	17927	-	17588288
West Bengal	1125	2	-	149	1	84	-	2055288	644	-	283183	218	227261	-	-	-	-	-	-	227261	-	-	6526557

Andhra Pradesh, Punjab, Haryana and Tamil Nadu (Fig. 2). Similar findings were observed in the *Rabi* season. Progressive farmers and input dealers are the most common platform to get technical advice from the farmers' point of view. Most of farmers depend heavily upon local advice, i.e., input dealers and progressive farmers. The public extension services by State Agricultural Universities and *Krishi Vigyan Kendras* are benefiting farmers. However, a low percentage

of farmers (varied among states) have been served by public extension services. The government sources are not as effective in their outreach to farmers. Farmers meet most of their information needs from other sources, of which farmer-to-farmer exchange is the most prominent, with more or less one-fourth of total agricultural households relying on progressive farmers.

**Input wise source of information:** Another critical issue is

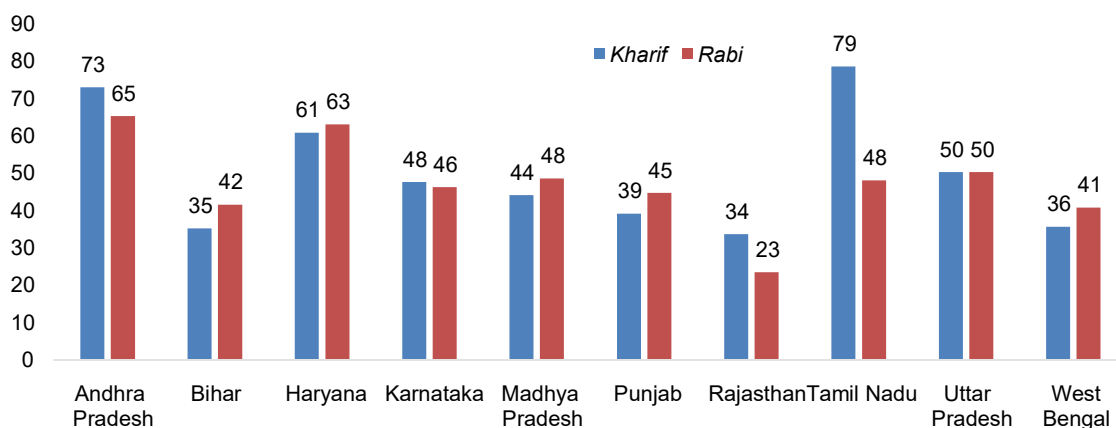
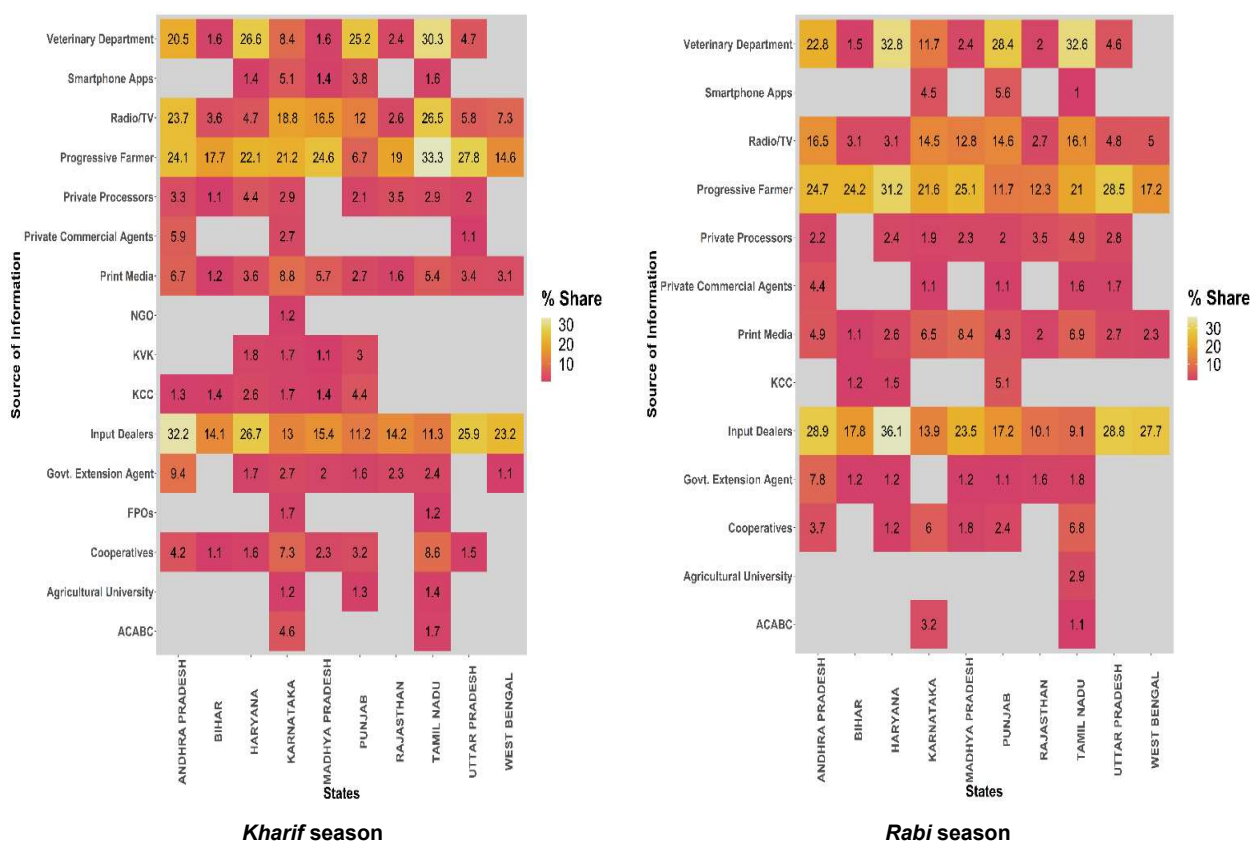


Fig. 1. Proportion (%) of the agricultural households who took technical advice regarding agricultural production



NGO: Non-Government Organization; KVK: *KrishiVigyanKendras*; KCC: Kisan Call Centre FPOs: Farmers Producers organizations; ACABC: Agri. Clinic and Agri. Business Centre

Fig. 2. Sources of information accessed by the agricultural households (multiple response %)

the choice of information that primarily led to determining the proximity, assured quality, and timely availability (Babu et al., 2013). Figure 3a and 3b provides information about the input-wise source of knowledge accessed by farmers across the states. For study, selected four major inputs, i.e., seed variety, plant protection, fertilizer application and harvesting for extension services.

**Information on seed Variety:** The results were expected, as progressive farmers dominated in providing information on available new seed varieties in markets. This source of information played a pioneering role among all the selected states of India. Information on seed varieties across states (Fig. 3a.) In Karnataka, Farmers Producer Organizations and Agricultural Clinic, and Agricultural Business Centres are important sources of information, with a share of 54.6 and 53.0 % of agricultural households on seed varieties, respectively. While during the *rabi* season (Fig. 3b), the primary source of information about the seed variety was gathered from Agricultural Clinic and Agricultural Business Centres (88%) and Progressive Farmers (76%). The public extension services like Government Extension Agents are

also actively participating in providing information to farmers of Andhra Pradesh (34.7%), Bihar (43.4%), Haryana (57.1%), Rajasthan (87.2%) and Madhya Pradesh (76 %), respectively during the *kharif* season. In contrast, in the case of *rabi* season, the proportion of the farmers was 26.8, 49.2, 91.1, 0.20, 40.8 and 85.7 %, respectively, for the above-said states of India.

**Information on plant protection:** The information on plant protection plays a significant role due to the sensitivity of the crop. Farmers from Madhya Pradesh, Punjab and Andhra Pradesh rely on the input dealers for information on plant protection. However, Agricultural Clinic and Agricultural Business Centres, and Kisan Call Centres are the preferable sources of information on plant protection in Haryana, Karnataka and Tamil Nadu (Fig 3a). Around 58 percent of agricultural households in Punjab and Tamil Nadu and about 46 percent in Uttar Pradesh preferred agricultural universities for information on plant protection during the *kharif* season. In *rabi* season, the pronounced source of information was FPOs in case of Punjab, Private commercial agents in Tamil Nadu. Most farmers also rely on the information given by the

**Table 2.** Crop and state-wise awareness of Minimum Support Price (MSP) (%)

State	Paddy	Jowar	Bajra	Maize	Ragi	Wheat	Barley
<i>Kharif</i> (July to December 2018)							
Andhra Pradesh	45.71	40.71	-	18.48	-	-	-
Bihar	31.62	0.41	11.95	23.63	-	-	-
Haryana	32.79	10.14	49.64	-	-	-	-
Karnataka	9.33	23.13	24.54	21.7	1.92	-	-
Madhya Pradesh	55.94	45.13	61.77	15.7	-	-	-
Punjab	52.13	-	89.42	57.32	-	-	-
Rajasthan	44.16	30.17	19.73	18.98	-	-	-
Tamil Nadu	32.39	0.22	-	8.4	22.52	-	-
Uttar Pradesh	31.59	29.63	37.39	38.19	-	-	-
West Bengal	47.10	100.00	-	9.75	-	-	-
<i>Rabi</i> (January to June 2019)							
Andhra Pradesh	48.6	42.86	-	35.37	3.8	-	-
Bihar	81.51	-	-	20.5	87	24.86	-
Haryana	44.06	-	100.00	100	-	63.12	-
Karnataka	13.3	9.86	18.63	16.78	9.15	43.76	-
Punjab	99.79	-	-	41.28	-	65.48	-
Rajasthan	-	-	-	-	-	35.18	33.10
Tamil Nadu	61.79	0.08	-	20.19	50.37	-	-
Uttar Pradesh	95.48	39.42	26.7	53.6	100.00	36.70	81.82
West Bengal	53.21	62.33	-	11.03	-	16.85	-
Madhya Pradesh	-	-	-	33.35	-	45.03	14.12

**Note:** Authors' calculation from NSSO 77<sup>th</sup> round



*Krishi Vigyan Kendras*, with a share of 66 per cent (Fig. 3b). But these sources of information are lesser effective in the remaining states.

**Fertilizer applications:** Input dealers, followed by progressive farmers, are the most reliable sources of information with variations across the regions. The proportion of farmers who rely on input dealers and progressive farmers for the fertilizer application was 36.8 and 41.9 per cent in Andhra Pradesh, about 22 per cent for each in case of Bihar and 22 and 32 per cent in case of Haryana during the *kharif* season. In *rabi* season, about one-half of the farmers belonging to Andhra Pradesh rely upon Agri clinics, about one-third of farmers from Bihar, about 56 per cent of farmers from Madhya Pradesh, and 61 per cent of the farmers from Rajasthan rely upon Agricultural universities to get the information about fertilizer application in different crops. However, agricultural households from Tamil Nadu, West Bengal, Karnataka and Madhya Pradesh rely on input dealers with 60.3 percent, 53.6 percent, 53.3 percent and 30.8 percent, respectively. But in the *rabi* season, only one-third of the farmers assessed this source of information. Further, the results revealed that public extension sources such as Government Extension Agents (26.5%) and *Krishi Vigyan Kendras* (23.7%) are working well in providing information on fertilizer application in Punjab *kharif* season and *rabi* season, the share was 3.69 and 7.8 per cent, respectively.

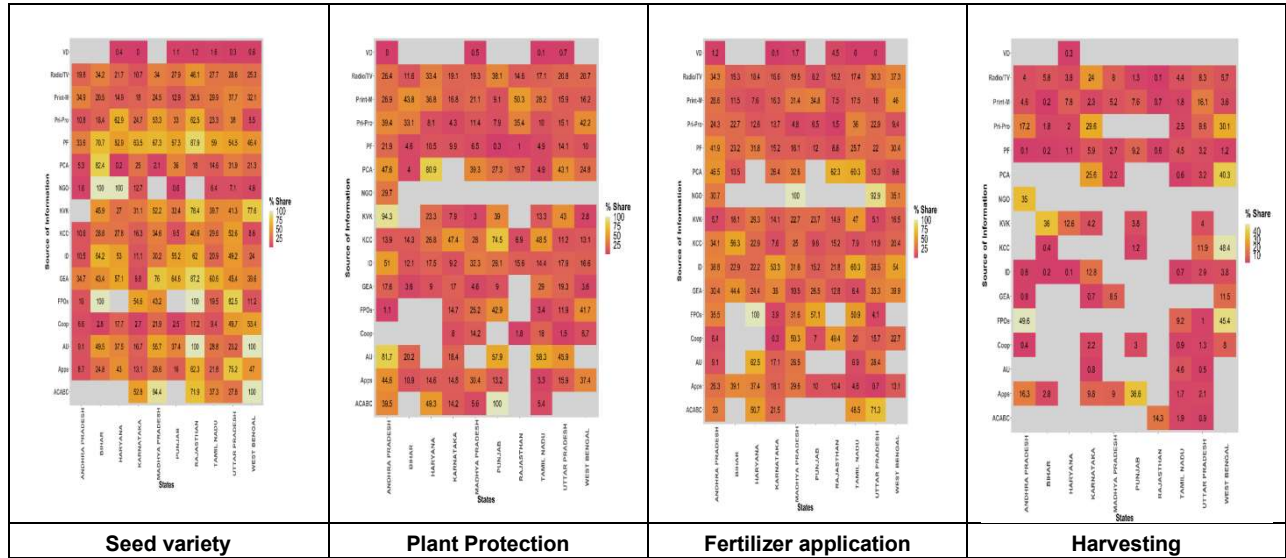
**Harvesting and marketing:** For this category of input, almost all the farmers across the states were unaware of the information on harvesting and marketing as the percent share of the farmers was relatively less across the states, which signifies that the experts from the public along with private sectors are still weak in providing information on marketing and harvesting which could help the farmers to decide, '*where and when to sell*' the agricultural commodities. The extension services of agricultural households from Andhra Pradesh, West Bengal and Tamil Nadu were primarily met by Farmers Producers Organizations, with a share of 49.6, 45.4 and 9.2 percent of total agricultural households, respectively, and about a similar proportion was observed in case of *rabi* season (Fig. 3a, Fig. 3b). Smartphone applications in Punjab (35.2%) and print media (16.2%) in Uttar Pradesh are significant sources of information about harvesting and marketing. In Karnataka, about 39 per cent of the farmers got information from private processors and Radio/TV during the *rabi* season. Still, none of the government extension agencies approached the farmers to give relevant information about the harvesting and sale patterns of different crops across the states.

In a nutshell, the region and input-specific extension

services are varied across states. The private players such as input dealers and progressive farmers actively provide information on seed varieties, plant protection and fertilizer applications. However, agricultural households are acquiring relevant knowledge of harvesting and marketing to a reasonable extent from public extension services. Smartphone Applications and *Kisan Call Centres* are emerging sources of information across the states.

**Reforming agricultural extension based on region-specific needs: A way forward:** With recent advancements in the agriculture sector, the role of extension activities is more pronounced. The farmers are associated with multiple activities related to the value chain and supply chains for further entrepreneurship development. Providing extension services to agricultural households may be a new challenge for the public sector. Unfortunately, the public sector has quite a limited role in agricultural extension services. Simultaneously, private extension services have been acknowledged recently, as they can provide need-based extension services to farmers. In addition, the private sector has the potential for context-based extension services. These reasons switched the agricultural households to private sectors. Apart from this, the share of research and extension from GVA has reduced over time. Only 0.54% of Agricultural Gross Domestic Product (AgGDP) was spent on research and extension during 2014-15 (Gulati et al. 2018). The study conducted by Fan et al., 2007 found the potential for increasing the investment in agricultural research and development. Demand-based extension systems considering different regions of India needs complete reorientation for utilizing the existing funds. The present study will help in this direction, as the paper has examined region-wise and source-wise awareness of MSP and their information sources of agricultural households in India.

As expected, government extension agency officials' outreach is relatively weaker than private service providers. The study's findings revealed that agricultural households across the states in India relied on different sources for extension services. Private players such as input dealers and progressive farmers are actively participating in providing information on seed varieties, plant protection and fertilizer applications. However, farmers are acquiring relevant knowledge of harvesting and marketing to a reasonable extent from public extension services. Other sources such as FPOs, Cooperatives, Private Processors, Smartphone Applications, and *Kisan Call Centres* also emerged in recent times due to more involvement of the farmers with these networks. These findings are highly crucial for policy decisions. For the development of any extension policy, the farmers across every part of the country should be



VD: Veterinary Department; Pri-Pro: Private Processors; PF: Progressive Farmers; PCA: Private commercial Agents; NGO: Non-Government Organization; KVK: KrishiVigyanKendras; KCC: KisanCall Centres; ID: Input Dealers; GEA: Government Extension Agents; FPOs: Farmers Producers organizations; Coop: Cooperative societies; AU: Agricultural Universities; Apps: Smart Phone Applications; ACABC: Agri. Clinic and Agri. Business Centre

Fig. 3a. Source wise and type of information accessed by the agricultural households during the Kharif season

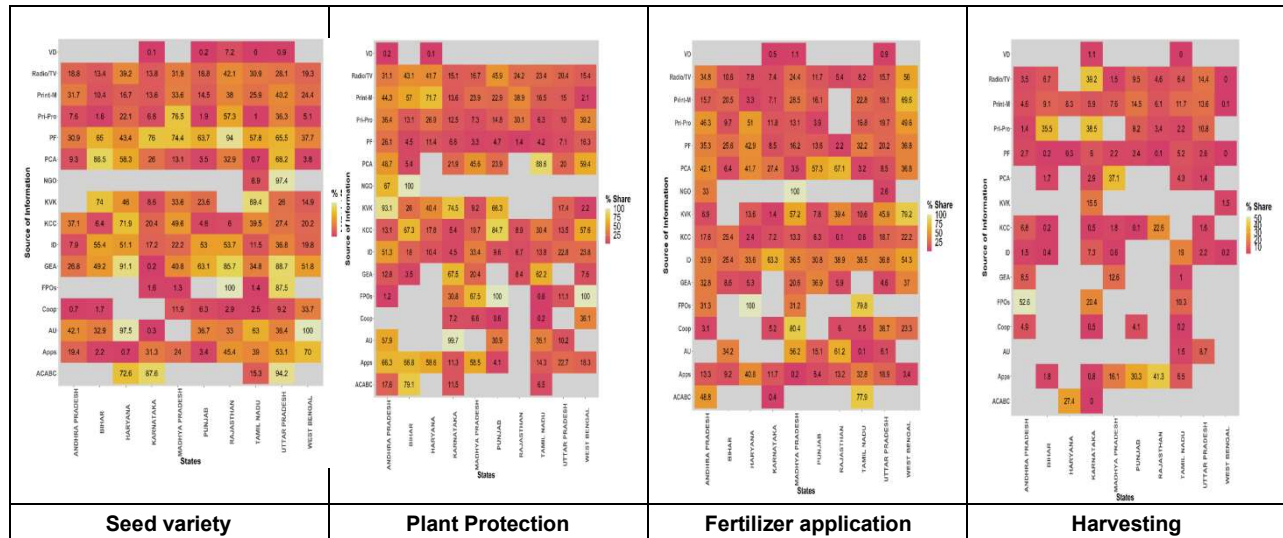


Fig. 3b. Source wise and type of information accessed by the agricultural households during the Rabi season

considered, as agricultural problems vary according to the geographical areas. This may help to utilize the funds as per the actual need of the agricultural households across the states. The extension system must be futuristic and evolve strategies to suit recent emerging issues. In this context, different institutional mechanisms must promote the public and private sectors to achieve inclusiveness and geographical coverage.

**CONCLUSIONS**

The agricultural households across the states in India relied on different sources for extension services. Private

players such as input dealers and progressive farmers are actively participating in providing information on seed varieties, plant protection and fertilizer applications. However, farmers are acquiring relevant knowledge of harvesting and marketing to a reasonable extent from public extension services. Other sources such as FPOs, cooperatives, private processors, smartphone applications, and Kisan Call Centres also emerged in recent times due to more involvement of the farmers with these networks. The extension system must be futuristic and evolve strategies to suit recent emerging issues. There is need to enhance public sector extension services through increased funding,

capacity building, and region-specific strategies to ensure inclusive and need-based support for agricultural households.

#### AUTHOR'S CONTRIBUTION:

All authors (KA, SK, PBB, AS) contributed to conceptualized the study. KA, SK PBB assisted for data analysis. All authors contributed to writing and reviewing the manuscript.

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# Water Quality Assessment of River Ganga in West Bengal based on Physico-Chemical and Bacteriological Parameters

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**Abstract:** The present study reveals the seasonal and spatial changes in physico-chemical as well as bacteriological parameters in the lower stretch of river Ganga at West Bengal. Water samples were collected from five different sampling stations viz., Serampore, Palta, Tribeni, Nabadwip and Berhampore on monthly basis for two consecutive years 2019-2020 and 2020-2021. Sixteen physico-chemical parameters-water temperature, total suspended solids, turbidity, total dissolved solids, electrical conductivity, pH, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, nitrate, nitrite, phosphate-phosphorous, total hardness, total alkalinity, sodium and potassium, and two bacteriological parameters - total coliform and fecal coliform were measured to assess the water quality of river Ganga at selected stations on a seasonal basis. Correlation analysis on these parameters displayed significant positive and negative correlations among themselves. Physico-chemical parameters didn't vary significantly at different sites, however seasonal variations were significant. The two bacteriological parameters were significantly different at different sampling sites but only total coliform significantly differed seasonally. DO, BOD, TH, TC and FC are critical parameters in this study. Among the five sampling stations, Berhampore and Serampore were more polluted in respect of bacteriological parameters. Human religious activities and mass bathing near Berhampore sampling station were responsible for high bacteriological pollution.

**Keywords:** ANOVA, Bacteriological parameters, Physico-chemical parameters, River Ganga, Water quality

River Ganga occupies a unique position in the cultural ethos of India. Millions of Hindus accept its water as sacred and count as river of faith, devotion and worship. River Ganga is considered to begin at confluence of Bhagirathi and Alaknanda at Devprayag in Garhwal Himalayas. It has total length of 2,525 km, raises in the western Himalayas in the Indian state of Uttarakhand, and flows south and east through the Gangetic Plain of North India and goes to Bay of Bengal through Bangladesh (Rai 2013). The entire pathway of the Ganga can be divided into three major stretches-upper, middle and lower. After flowing through the Sivalik hills, the Ganga enters plains at Haridwar. Then it flows southwards, passes through the plains of Uttar Pradesh and enters Bihar in the Rohtas district. After Bihar, the Ganga enters West Bengal and flows towards south and nearly 40 km below at Farakka, it divides into two streams. The left stream flows eastwards into Bangladesh and the right stream (Bhagirathi) continues to flow south through West Bengal (Paul 2017). The Bhagirathi flowing west and south-west of Kolkata is known as Hooghly. After reaching Diamond Harbour, it attains a southward direction and is split into two arms before reaching the Bay of Bengal (Rahaman 2009). The pathway of Ganga from Varanasi to Ganga Sagar is referred as its lower segment or stretch (GRB EMP 2010).

The Ganga was ranked among the five most polluted rivers of the world in 2007 (Rai 2013). The quality of river water is being degraded due to rapid population growth,

agricultural and industrial developments, making it unsuitable for various uses (Singh et al., 2016). The river is also a site for religious bathing (Kanvar mela, Kumbh mela etc.), idol immersion, washing and watering of animals and the disposal corpses. Tourism is another important factor that contributes higher pollution load. The river supports 400 million people and is worshipped by more than one billion of countries population (Das and Taminga 2012). Therefore, comprehensive monitoring of river water quality is necessary to safeguard public health and protect the vulnerable and valuable fresh water resource (Singh 2014).

Physico-chemical characteristics of river water affect the biological characteristics and indicate the status of river water quality. Water temperature influences the pH, alkalinity and DO of the water (Matta 2014). Turbidity influences the light penetration inside water affecting the aquatic life (Tambekar et al., 2013). pH affects aquatic organisms as most of the metabolic activities are pH dependent (Kumar et al., 2011). Dissolved oxygen, biochemical oxygen demand and chemical oxygen demand are three of the most important chemical parameters for water quality analysis. Alkalinity in water sample implies the presence of hydroxides, bicarbonates, carbonates ions (Kamboj and Kamboj 2019). The assessment of bacteriological parameters like total coliform and fecal coliform is very important as presence of pathogenic bacteria in water body can cause several water-borne diseases in both human and animals (Panda et al.,

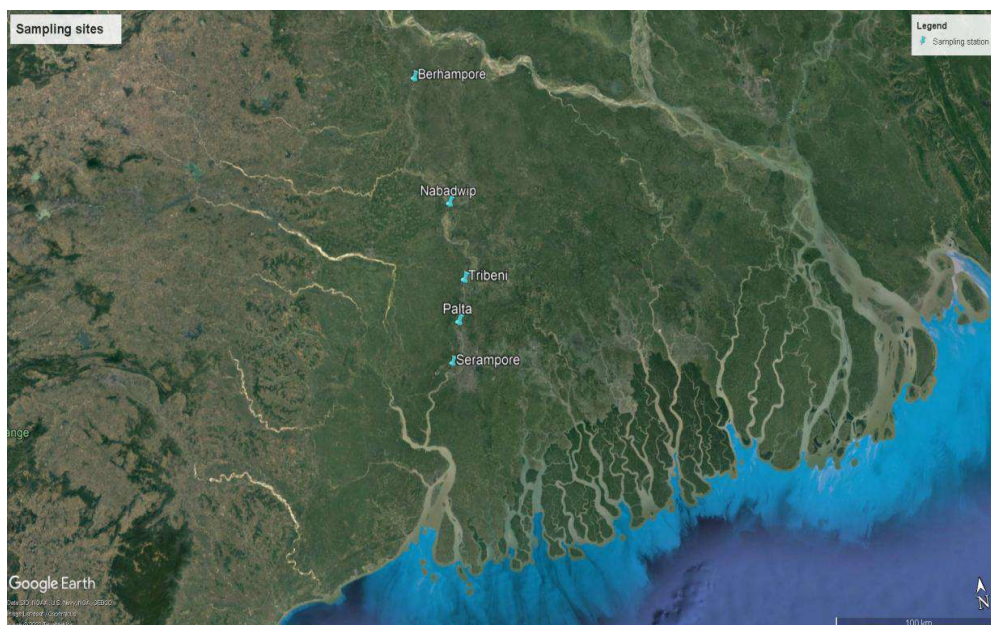
2018). Open defecation in the river bed, excreta of animals, burning and throwing of dead bodies, domestic waste water and biomedical wastes are the major sources of bacterial contamination (Panda et al., 2017).

Many authors assessed water quality of river Ganga and pointed out BOD, COD, TH, TSS and FC as critical parameters due to industrial pollution, sewage and human activities (Khare et al., 2011, Singh et al., 2016, Khan et al., 2017). Most of the earlier works on water quality of river Ganga were done in the middle stretch of Ganga (Tripathi et al., 2014, Matta 2014, Singh et al., 2016, Chauhan and Bhardwaj 2018). Very little research work has been done on the water quality in the lower stretch of the Ganga, particularly in West Bengal. Hence, the present study was undertaken to assess the impact of seasonal variation on physico-chemical and bacteriological parameters of the Ganga river water at its lower stretch. The study will provide a clear picture about the Ganga river which is very close to meet the Bay of Bengal.

#### MATERIAL AND METHODS

**Study area:** The present study was carried out in the lower stretch of the Ganga, known as Bhagirathi-Hooghly in West Bengal to examine water quality status. Water samples were collected from five sampling sites i.e. Serampore (22.76°N, 88.34°E), Palta (22.77°N, 88.34°E), Tribeni (22.98°N, 88.40°E), Nabadwip (23.39°N, 88.37°E) and Berhampore (24.11°N, 88.25°E). The sampling sites (Fig. 1) were populated areas with high domestic and industrial effluents

and come under used based class B and C of surface water in India according to Water Quality Standards of CPCB updated on 23<sup>rd</sup> October, 2019 (<https://cpcb.nic.in/water-quality-criteria/>). Serampore station is located on the right bank of the river Ganga in Hooghly district. It is very busy navigation route connecting major cities of either side of the river banks. The major polluting source comes from the domestic waste water through big drains. Palta is located on left bank of the river Ganga in the North 24 Parganas district. Some temples are situated near station and a big city Barrackpore is located in its close proximity. Major pollutants come from bathing, washing and effluents from drains. Tribeni station is located on the right bank of the river in Hooghly district near the burning Ghat of Tribeni. The name 'Tribeni' is believed to be originated from divergence of three historical rivers- Kunti, Ganga and Saraswati (mythical). Domestic sewage, religious bathing, washing and burning corpse are the major sources of pollutants. Nabadwip is located on the left bank of Ganga River in the district of Nadia. It is an important place for religious purpose with many big temples situated all over the city. Sampling station was located near Monipur Ghat. Bathing and religious rituals are the major pollution sources. Berhampore is one of the biggest cities in West Bengal located in Murshidabad district. Berhampore sampling station was on the left bank of the river near College Ghat of Berhampore. Although an electric crematorium is situated in this Ghat, many open-air cremations are done regularly and the corpse are thrown into the river. Many drains discharge their waste water without any treatment near the sampling station.



**Fig. 1.** Location of sampling sites in the lower stretch of river Ganga (Source: ©Google Earth)

**Sample collection and storage:** The water samples were collected at monthly interval throughout the year for duration of two years i.e. October 2019- September 2021 from the mentioned sampling sites. Water samples were collected at a depth of 1.0-1.5 m using a clean plastic bucket, transferred to clean plastic bottles and transported to the laboratory on ice and stored in refrigerator (4-6 °C) till analysis.

**Physico-chemical and bacteriological analysis:** All the parameters were analyzed according to the Standard Methods of American Public Health Association (23<sup>rd</sup> Edition 2017) (Table 1). Some physico-chemical parameters were recorded on the spot like water temperature (WT), pH, and dissolved oxygen (DO). Other parameters were analyzed in the laboratory.

**Statistical analyses:** Distribution of data was analyzed by Normality tests. Correlations among water parameters were estimated by non-parametric Spearman's rank correlation. Significant variations in water parameters due to sites and seasons were assessed by univariate ANOVA of log transformed values, followed by Tukey's post hoc test ( $\alpha < 0.05$ ). The statistical tests were performed in the SPSS software.

## RESULTS AND DISCUSSION

**Physical parameters:** The average water temperature varied from a minimum 21 °C at Berhampore during winter to a maximum 31 °C at Palta during rainy season (Table 2). Seasonally, the values of WT were highest in rainy season

**Table 1.** Methods used for physico-chemical and bacteriological parameters

Parameter	Unit	Method of analysis/ Instrument
Water Temperature (WT)	°C	Glass thermometer
pH	Unit less	pH meter and pH electrode
Dissolved Oxygen (DO)	mg/L	Winkler's Method
Electrical Conductivity (EC)	µS/cm	Conductivity meter and conductivity electrode
Turbidity	NTU	Turbidity meter
Total Suspended Solids (TSS)	mg/L	Gooch crucible method (Gravimetric)
Total Dissolved Solids (TDS)	mg/L	Drying method (Gravimetric)
Chemical Oxygen Demand (COD)	mg/L	Open reflux method
Biochemical Oxygen Demand (BOD)	mg/L	3 days' BOD measurement method
Nitrate (NO <sub>3</sub> -N)	mg/L	Cadmium Reduction Method (Spectrophotometric)
Nitrite (NO <sub>2</sub> -N)	mg/L	Spectrophotometric method
Phosphate-Phosphorus (PO <sub>4</sub> -P)	mg/L	Stannous chloride method (Spectrophotometric)
Sodium (Na)	mg/L	Flame photometer
Potassium (K)	mg/L	Flame photometer
Total Alkalinity (TA)	mg/L	Titrimetric method
Total Hardness as CaCO <sub>3</sub> (TH)	mg/L	Titrimetric method
Total Coliform (TC)	MPN/100 ml	Most probable number method
Fecal Coliform (FC)	MPN/100 ml	Most probable number method

**Table 2.** Physical parameters (mean ± standard deviation) at different sites in different seasons

Station	Season	WT (°C)	TSS (mg/L)	TDS (mg/L)	EC (µs/cm)	Turbidity (NTU)
Serampore	Summer	29±3	45±29	159±55	338.4±75.1	51.5±28.3
	Rainy season	30±2	128±59	150±31	254.5±18.1	123.1±51.5
	Winter	23±3	50±32	201±44	339.9±40.5	53.3±32.7
Palta	Summer	29±2	89±50	156±61	329.7±56.9	82.1±46.8
	Rainy season	31±2	134±93	146±26	251.3±20.2	157.5±60.5
	Winter	23±4	63±37	196±29	336.8±50.2	60.1±46.4
Tribeni	Summer	29±2	53±21	186±37	354.5±65.6	51.3±18.8
	Rainy season	31±1	131±56	151±22	258.6±20.1	120.7±75.7
	Winter	22±3	85±45	200±33	344.3±49	74.5±48.7
Nabadwip	Summer	29±2	55±25	165±60	347.3±67.3	47.6±18.5
	Rainy season	30±1	183±81	146±17	235.8±16	168.1±86.9
	Winter	22±3	63±33	190±34	322.4±57.7	57.1±33.2
Berhampore	Summer	28±3	29±20	162±56	337.5±68.5	39.5±20.7
	Rainy season	30±1	217±157	148±22	251.4±23.3	162.2±81.2
	Winter	21±3	58±30	193±24	347.7±29.6	52.1±36.6

followed by summer and winter. The average turbidity varied from a minimum 39.5 NTU during summer at Berhampore to a maximum of 168.1 NTU during rainy season at Nabadwip. Previously, lower and higher range of turbidity have been reported from river Ganga – 28.66 to 63.66 NTU (Tripathi et al., 2014, Allahabad) and 126.29 to 165.56 NTU (Matta 2014, Uttarakhand) – the higher value was during monsoon. The higher number of suspended particles present in water results in higher turbidity. Both TSS and turbidity were higher in rainy season. The average TSS was maximum (217 mg/L) during rainy season at Berhampore and minimum (29 mg/L) during summer at Berhampore. Due to the surface run off and dissolving clay matter of the river banks, TSS always tends to be higher in rainy season.

Total dissolved solids indicate the presence of ions in water. The average TDS varied from 146 mg/L at Palta during rainy season to 201 mg/L at Serampore during winter. This is below the permissible limit as per BIS standard (500 mg/L). The average value of electrical conductivity was maximum (354.5 µS/cm) during summer at Tribeni and minimum (235.8 µS/cm) during rainy season at Nabadwip. The values are within the acceptable range of 20-500 µS/cm (Vijayakumar and Amanchi 2024). TDS and EC are closely associated with each other as the dissolved ions are responsible for greater conductivity in water sample. TDS and EC were lowest in rainy season due to dilution in water and highest in winter, similar to Yadav and Srivastava (2011) at Gazipur, Uttar Pradesh.

**Chemical parameters:** pH varied from minimum 7.84 at Tribeni during rainy season to maximum 8.30 at Berhampore during winter (Table 3). Joshi et al. (2009) and Kumar et al. (2018) also reported slightly alkaline nature of the Ganga River water at Haridwar. pH value becomes lower in rainy season due to heavy deposition of slightly acidic rain water. Average dissolved oxygen, biological oxygen demand and chemical oxygen demand varied from minimum 4.7 mg/L during rainy season at Palta, 2.20 mg/L during rainy season at Berhampore and 9.09 mg/L during winter at Nabadwip, respectively, to maximum 8.9 mg/L during winter at Berhampore, 3.52 mg/L at Tribeni during winter and 12.13 mg/L at Tribeni during rainy season, respectively. DO was highest in winter followed by summer and rainy season, similar to Ramganga River at Kumaun Himalaya (Verma 2013), and fell below the BIS standard of minimum 5 mg/L during rainy season. Solubility of oxygen in water decreases with increase in temperature. BOD exceeded the BIS standard (3 mg/L) in winter and indicates polluted status.

Nitrate, nitrite and phosphate varied from minimum 0.45 mg/L during summer at Nabadwip, 0.02 mg/L during summer at Tribeni and 0.06 mg/L during winter at Serampore,

**Table 3.** Chemical parameters at different sites in different seasons (mean ± standard deviation)

Station	Season	pH	DO (mg/L)	BOD (mg/L)	COD (mg/L)	NO <sub>3</sub> -N (mg/L)	NO <sub>2</sub> -N (mg/L)	PO <sub>4</sub> -P (mg/L)	TH (mg/L)	TA (mg/L)	Na (mg/L)	K (mg/L)
Serampore	Summer	8.05±0.2	6.4±0.6	2.76±0.7	9.96±1.7	0.47±0.1	0.03±0	0.09±0	137.52±27.8	132.91±26.9	16.3±4.3	4.3±0.6
	Rainy season	7.88±0.2	5.1±0.5	2.45±0.8	11.01±1.5	0.55±0.2	0.06±0.1	0.13±0.1	111.72±10.4	105.87±15.9	14±5.4	4.6±0.8
	Winter	8.26±0.2	8.6±1.9	3.38±1.2	10.59±2.9	0.48±0.1	0.1±0.1	0.06±0	157.60±12.6	151.46±12.3	17.9±2.8	4.2±0.6
Palta	Summer	8.14±0.2	6.2±1	2.97±0.39	10.53±3	0.49±0.13	0.06±0.1	0.08±0.03	138.53±25.52	135.48±26	17.4±3.6	4.3±0.6
	Rainy season	7.92±0.17	4.7±0.4	2.48±0.4	11.08±1.52	0.55±0.19	0.04±0.02	0.13±0.05	117.25±17.09	108.72±10.18	13.9±5.7	4.8±0.8
	Winter	8.19±0.12	8.3±1.5	3.37±1.36	11.79±3.89	0.5±0.08	0.05±0.05	0.06±0.02	152.10±15.73	153.74±15.8	17.3±3	4.0±0.3
Tribeni	Summer	8.18±0.32	6.9±0.9	3.03±0.52	11.04±2.08	0.51±0.18	0.03±0.01	0.08±0.03	144.42±21.51	137.11±30.73	17.2±4.6	4.5±0.6
	Rainy season	7.84±0.12	5.2±0.4	2.82±0.62	12.13±2.1	0.59±0.18	0.05±0.06	0.10±0.05	117.13±11.5	106.68±9.22	15.4±6.3	4.7±0.9
	Winter	8.17±0.13	8.3±1.4	3.52±1.37	10.76±2.1	0.49±0.11	0.06±0.04	0.07±0.03	151.22±17.84	154.21±15.12	18.7±2.5	4.4±0.7
Nabadwip	Summer	8.20±0.2	6.8±0.6	2.55±0.67	9.41±1.2	0.45±0.08	0.02±0.02	0.07±0.03	134.12±25.72	133.72±28.19	16.9±3.6	3.9±0.4
	Rainy season	7.90±0.14	5.7±0.3	3.10±0.81	11.71±2.68	0.52±0.15	0.04±0.03	0.12±0.05	121.30±33.83	101.51±7.43	12.2±3.5	4.0±0.7
	Winter	8.29±0.23	8.5±1.5	3.06±0.95	9.09±1.54	0.46±0.08	0.03±0.05	0.08±0.02	143.36±17.05	146.30±14.61	17.2±2.4	4.0±0.7
Berhampore	Summer	8.16±0.3	6.8±1.0	2.67±0.96	10.14±1.69	0.48±0.1	0.03±0.02	0.16±0.22	142.40±27.62	127.98±24.84	16.6±4.0	4.1±0.5
	Rainy season	7.93±0.11	5.9±0.5	2.20±0.64	11.02±2.0	0.51±0.16	0.02±0.01	0.11±0.04	110.20±5.31	107.38±8.66	11.6±5.5	4.8±0.9
	Winter	8.30±0.21	8.9±1.0	3.05±0.62	9.89±2.03	0.47±0.08	0.03±0.01	0.07±0.04	150.03±8.32	153.46±17.03	17.1±3.1	3.8±0.3

respectively, to maximum 0.58 mg/L during rainy season at Tribeni, 0.10 mg/L at Serampore during winter and 0.13 mg/L at Serampore during rainy season, respectively. Slightly higher values ( $\text{NO}_3\text{-N}$ : 0.35 – 0.81 mg/L,  $\text{PO}_4\text{-P}$ : 0.27 – 0.75 mg/L) have been reported from river Ganga at Allahabad (Tripathi et al., 2014). High quantity of these nutrients is harmful for any water body as it causes algal bloom and hampers the indigenous aquatic life.

Total hardness was maximum (157.60 mg/L) during winter at Serampore and minimum (110.20 mg/L) during rainy season at Berhampore. Water with  $\text{TH} > 75$  mg/L is considered hard water (Kumar et al., 2010). Total alkalinity varied from minimum 101.51 mg/L at Nabadwip during rainy season to maximum 154.21 mg/L at Tribeni during winter. TH and TA were higher in winter followed by summer and became lowest in rainy season due to deposition of rain water.

Sodium varied from minimum 11.6 mg/L during rainy season at Berhampore to maximum 18.7 mg/L during winter at Tribeni. Potassium was maximum (4.8 mg/L) during rainy season at Berhampore and minimum (3.8 mg/L) during winter at Berhampore. These values are much higher than the range of Na (0.40-0.90 mg/L) and K (1.10-1.40 mg/L) reported by Singh et al. (2016) for Ganga River at Varanasi.

**Bacteriological parameters:** Total coliform ranged from 3350 to 258500 MPN/100 ml in summer, 15500 to 490000 MPN/100 ml in rainy season and 4650 to 175000 MPN/100 ml in winter (Table 4). Fecal coliform ranged from 1550 ml to

156500 MPN/100 ml in summer, 6500 to 125000 MPN/100 ml in rainy season and 2650 to 109500 MPN/100 ml in winter. These values are much lower than those from Ganga River at Haridwar (Chauhan and Bhardwaj 2018, Kumar et al., 2018), where heavy influx of pilgrims is common. However, they exceeded the tolerance limit for inland surface water subject to pollution (IS: 2296-1982) which is set at 5000 MPN/100 ml for TC and 2500 MPN/100 ml for FC (Environmental Standards 2014). TC and FC were higher in rainy season followed by summer and winter. Coliform bacteria tend to be lower in winter as water temperature remains lower than the optimum temperature for bacterial growth.

**Correlation coefficients ( $\rho$ ) among various water quality parameters:** Water quality parameters displayed significant positive and negative correlations among themselves (Table 5). pH had strong positive correlation with EC, DO, TH, TDS, TA and Na, while negative correlation with TSS,  $\text{PO}_4\text{-P}$ ,  $\text{NO}_3\text{-N}$ , K, TC, FC, COD and turbidity. Water temperature was strongly positively correlated with TSS,  $\text{PO}_4\text{-P}$ , K, TC, FC and turbidity, and negatively with pH, EC, DO, BOD, TH, TDS, TA and Na. These correlations indicate response of other parameters towards seasonal changes in water temperature and rain. For instance, both DO and BOD decreased during rainy season and increased during winter displaying strong positive correlation. TC and FC showed strong positive correlation with WT, TSS and  $\text{PO}_4\text{-P}$ , indicating that increased TSS and  $\text{PO}_4\text{-P}$  at high temperature boosts the bacterial multiplication. Dilution of water during rainy season lowered

**Table 4.** Bacteriological parameters (range) at different sites in different seasons

Station	Season	Total coliform (MPN/100 mL)	Faecal coliform (MPN/100 mL)
Serampore	Summer	17000-240000	14000-130000
	Rainy season	39500-260000	19500-100000
	Winter	4900-164500	3300-101500
Palta	Summer	50000-240000	13000-105000
	Rainy season	40000-240000	11500-109500
	Winter	18000-175000	10000-95000
Tribeni	Summer	13000-170000	7900-92000
	Rainy season	15500-130000	6500-79000
	Winter	5600-55000	1300-36000
Nabadwip	Summer	8500-258500	5650-156500
	Rainy season	25000-490000	12000-118500
	Winter	4650-175000	2650-109500
Berhampore	Summer	3350-205000	1550-94500
	Rainy season	50000-270000	26500-125000
	Winter	21000-150000	6900-104500



**Table 5.** Spearman's rank correlation coefficients ( $\rho$ ) among various water quality parameters

Parameters	pH	EC	DO	BOD	TSS	NO <sub>3</sub> -N	NO <sub>2</sub> -N	NO <sub>3</sub> -P	TH	TDS	Na	TA	K	TC	FC	COD	Turbidity
	(°C)	( $\mu$ s/cm)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(MPN/100ml)	(MPN/100ml)	(mg/L)	(NTU)
WT (°C)	-.634**	-.527**	-.816**	-.334**	.395**	.128	-.123	.450**	-.527**	-.424**	-.408**	-.691**	.317**	.318**	.263**	.095	.450**
pH	1.000	.587**	.732**	.199	-.577**	-.341**	.038	-.469**	.355**	.434**	.456**	.675**	-.238**	-.411**	-.405**	-.218	-.562**
EC ( $\mu$ s/cm)		1.000	.703**	.148	-.514**	-.135	.001	-.166	.697**	.707**	.579**	.748**	.000	-.376**	-.337**	.012	-.603**
DO (mg/L)			1.000	.277**	-.538**	-.200	-.059	-.395**	.637**	.545**	.460**	.779**	-.232**	-.374**	-.330**	-.141	-.632**
BOD (mg/L)				1.000	-.059	.037	.301**	-.227**	.261**	-.008	.216	.219	-.102	-.056	-.049	.265**	-.094
TSS (mg/L)					1.000	.235**	.039	.289**	-.336**	-.241**	-.413**	-.465**	.024	.265**	.218	.252**	.845**
NO <sub>3</sub> -N (mg/L)						1.000	-.095	.311**	-.010	-.153	-.184**	-.306**	-.041	-.023	.039	.230	.114
NO <sub>2</sub> -N (mg/L)							1.000	-.116	-.004	.050	.112	.066	.096	.018	-.080	.198	.128
PO <sub>4</sub> -P (mg/L)								1.000	-.125	-.112	-.205	-.378**	.257**	.280**	.243**	.198	.257**
TH (mg/L)									1.000	.553**	.429**	.661**	-.012	-.265**	-.230**	-.067	-.440**
TDS (mg/L)										1.000	.413**	.672**	.072	-.218**	-.214**	-.080	-.309**
Na (mg/L)											1.000	.661**	.438**	-.210**	-.210**	.152	-.456**
TA (mg/L)												1.000	.030	-.330**	-.144	-.502**	
K (mg/L)													1.000	.049	.214	.011	
TC (MPN/100 mL)														1.000	.908**	-.012	.328**
FC (MPN/100 mL)															1.000	.036	.258**
COD (mg/L)																1.000	.152

\*\*=  $p \leq 0.01$ ; \* =  $p \leq 0.05$ ; Bold numbers indicate significant correlation

**Table 6.** F-value (significance) showing effect of sites and seasons on water parameters

Dependent parameter	Site	Season	Site * Season
WT (°C)	.932 (.448)	121.978 (.000)	.277 (.972)
pH	.703 (.591)	33.320 (.000)	.462 (.880)
EC (µs/cm)	.441 (.779)	45.694 (.000)	.227(.985)
Turbidity (NTU)	.780 (.541)	41.798 (.000)	.950(.480)
DO (mg/L)	2.110 (.085)	101.647 (.000)	.417 (.908)
BOD (mg/L)	.997 (.412)	6.513 (.002)	.644 (.739)
COD (mg/L)	1.337 (.261)	3.139 (.047)	.654 (.730)
TSS (mg/L)	.753 (.559)	34.538 (.000)	1.955 (.059)
TDS (mg/L)	.440 (.780)	15.163 (.000)	.208 (.989)
NO <sub>3</sub> N (mg/L)	.597 (.666)	3.039 (.052)	.072 (1.000)
NO <sub>2</sub> N (mg/L)	1.937 (.110)	1.645 (.198)	.727 (.668)
P (mg/L)	.582 (.676)	5.934 (.004)	.900 (.520)
TH (mg/L)	.186 (.945)	31.984 (.000)	.527 (.834)
TA (mg/L)	.351 (.843)	58.336 (.000)	.155 (.996)
Na (mg/L)	.823 (.513)	11.609 (.000)	.270 (.974)
K (mg/L)	2.794 (.030)	6.515 (.002)	.742 (.654)
TC (MPN/100 mL)	3.158 (.017)	6.476 (.002)	1.363 (.221)
FC (MPN/100 mL)	2.485 (.048)	2.707 (.071)	.838 (.571)

Na leading to low EC and TDS indicating strong positive correlation.

Physico-chemical parameters didn't vary significantly at different sampling sites, except for K, but bacteriological parameters were significantly different site-wise (Table 6). Post-hoc test (Table 7) resulting into homogenous subsets showed significantly higher mean K in Tribeni than in Nabadwip. Mean TC at Serampore was significantly higher than at Tribeni, while both TC and FC were significantly higher at Berhampore than at Tribeni. All the physico-chemical parameters except nitrate and nitrite differed significantly season-wise. Post-hoc test placed WT, DO, TH and TA into three subsets with significantly different values in all the three seasons (Table 8). WT, turbidity, TSS, PO<sub>4</sub>-P and K were significantly higher while EC, pH, DO, BOD, TDS, TH, TA and Na were significantly lower in rainy season than in other seasons. Winter season differed significantly from other seasons in terms of maximum pH, DO, BOD, TDS, TH, TA and Na, and minimum WT, PO<sub>4</sub>-P and K. Among bacteriological parameters, only total coliform varied significantly in different seasons with significantly higher value in rainy season.

**Table 7.** Mean values of significantly different water parameters across five sites

Parameter	Sites				
	Tribeni	Nabadwip	Palta	Serampore	Berhampore
K (mg/L)	4.55±0.15 <sup>b</sup>	3.95±0.11 <sup>a</sup>	4.33±0.13 <sup>ab</sup>	4.35±0.14 <sup>ab</sup>	4.23±0.15 <sup>ab</sup>
TC (MPN/100 mL)	46858.33±8437.96 <sup>a</sup>	84229.17±22124.53 <sup>ab</sup>	96331.25±12599.05 <sup>ab</sup>	109183.33±15444.98 <sup>b</sup>	110943.75±16037.19 <sup>b</sup>
FC (MPN/100 mL)	25354.1±4980.73 <sup>a</sup>	38533.33±8629.10 <sup>ab</sup>	42947.92±6520.04 <sup>ab</sup>	47575±7232.36 <sup>ab</sup>	55031.25±7572.30 <sup>b</sup>

Small superscript alphabets represent homogenous subsets after Tukey's post-hoc test ( $\alpha=0.05$ ).

**Table 8.** Mean values of significantly different water parameters across three seasons

Parameter	Seasons		
	Winter	Summer	Rainy
WT (°C)	22.28±0.49 <sup>a</sup>	28.73±0.35 <sup>b</sup>	30.28±0.23 <sup>c</sup>
DO (mg/L)	8.54±0.23 <sup>c</sup>	6.61±0.13 <sup>b</sup>	5.31±0.10 <sup>a</sup>
TH (mg/L)	150.86±2.32 <sup>c</sup>	139.40±3.90 <sup>b</sup>	115.52±2.84 <sup>a</sup>
TA (mg/L)	151.83±2.30 <sup>c</sup>	133.44±4.14 <sup>b</sup>	106.03±1.65 <sup>a</sup>
EC (µs/cm)	338.22±7.09 <sup>b</sup>	341.46±10.12 <sup>b</sup>	250.33±3.20 <sup>a</sup>
Turbidity (NTU)	59.44±6.14 <sup>a</sup>	54.38±4.88 <sup>a</sup>	146.33±11.32 <sup>b</sup>
pH	8.24±0.03 <sup>b</sup>	8.15±0.04 <sup>b</sup>	7.89±0.02 <sup>a</sup>
BOD (mg/L)	3.28±0.17 <sup>b</sup>	2.80±0.10 <sup>a</sup>	2.61±0.11 <sup>a</sup>
COD (mg/L)	10.43±0.42 <sup>a</sup>	10.22±0.31 <sup>b</sup>	11.39±0.31 <sup>b</sup>
TSS (mg/L)	63.65±5.66 <sup>a</sup>	54.3±5.62 <sup>a</sup>	158.35±15.51 <sup>b</sup>
TDS (mg/L)	196.05±5.04 <sup>b</sup>	165.75±8.31 <sup>a</sup>	148.1±3.64 <sup>a</sup>
PO <sub>4</sub> -P (mg/L)	0.07±0.00 <sup>a</sup>	0.09±0.02 <sup>ab</sup>	0.12±0.01 <sup>b</sup>
Na (mg/L)	17.64±0.43 <sup>b</sup>	16.87±0.61 <sup>b</sup>	13.40±0.83 <sup>a</sup>
K (mg/L)	4.07±0.08 <sup>a</sup>	4.21±0.09 <sup>a</sup>	4.57±0.13 <sup>b</sup>
TC (MPN/100 mL)	63628.75±8534.88 <sup>a</sup>	83998.75±12323.29 <sup>a</sup>	120900±14590.22 <sup>b</sup>

Small superscript alphabets represent homogenous subsets after Tukey's post-hoc test ( $\alpha=0.05$ ).

## CONCLUSIONS

The present study analyzed total 18 physico-chemical and bacteriological parameters in the lower stretch of the Ganga. These parameters varied significantly season-wise. DO, BOD, TH, TC and FC were identified as critical parameters in this stretch of the Ganga. Rainy season was more polluted with heavy increase in both TC and FC leading to drastic fall in DO. BOD was alarmingly high in winter. Among the five sampling stations, Berhampore and Serampore were more polluted in respect of bacteriological parameters owing to discharge of waste water without prior treatment and throwing of corpses. The water quality of river Ganga, considered as most sacred, is unfit for use without proper treatment. Therefore, the role of Sewage Treatment Plants (STPs) plays very important part to revive the water quality. Both Central and State Governments have taken some major steps to purify the water and recover the holy status of the Ganges. Regular monitoring is essential to keep a check on water quality and also to maintain the aesthetical value.

## ACKNOWLEDGEMENTS

We thank the UGC-DRS and DST-FIST supported Department of Botany, Visva-Bharati for providing research facilities and administrative help.

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# Assessing the Sustainability of Farmer Producer Companies (FPCs) in Western Himalayan Region

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**Abstract:** This study assesses the sustainability and future prospects of Farmer Producer Companies (FPCs) in Himachal Pradesh (HP). According to the secondary data collected from the Horticulture Development Project (funded by World Bank), National Bank for Agriculture and Rural Development (NABARD), Shimla and Small Farmers Agribusiness Consortium (SFAC) there are 216 FPCs registered, with the highest numbers in Shimla (36), Mandi (33), Sirmaur (31) and Kangra (26), accounting for over 50% of the state's FPCs. Most FPCs were formed between 2021 and 2023, with a linear growth rate of 35.11 percent annually since 2015. The study evaluates the activities of FPCs, revealing that 53 percent focus on horticultural crops, 41 percent on agricultural and allied activities, and fewer on handicrafts, dairy, fishery, and forestry. Using the ARIMA model, it forecasts the registration of 500 more FPCs by 2030. The study recommends training programs, sustainable harvesting, marketing techniques, and NTFP diversification to enhance the sustainability of FPCs. The Himachal Pradesh government's initiatives in registering well-established natural FPCs further promote sustainability.

**Keywords:** Farmer Producer Companies (FPCs), Linear growth rate (LGR), Sustainable Development Goals (SDGs), ARIMA model

The concept of producer companies, introduced in India in 2002 as part IXA of the Companies Act 1956 under economist Y K Alagh, offered Indian farmers a new path towards livelihood sustainability (Jose 2018). The first producer company was registered in 2004, and the number grew to 15,948 by 2021, with a significant increase in registrations during the financial years 2020 and 2021 (Padaliya et al., 2022). According to the Union Ministry of Corporate Affairs, there were 16,000 Farmer Producer Companies (FPCs) in the country as of February 2023, with 65 per cent of these being registered in the past three years (2020-21, 2021-22, 2022-23) (DTE 2023). In December 2019, Small Farmers' Agri-business Consortium (SFAC) released a "Strategy Paper for Promotion of 10,000 Farmer Producer Organizations (FPOs)", which was followed in July 2020 by the release of Operational Guidelines by the Department of Agriculture, Co-operation & Farmers' Welfare (SFAC 2019; DACFW 2020; Govil and Neti 2021). The scheme aims to provide holistic and broad based supportive ecosystem to facilitate development of vibrant and sustainable income oriented farming and for overall socio-economic development and wellbeing of agrarian communities (DACFW 2020). When the Scheme for Promotion of 10,000 FPOs has been announced since then the large numbers of producer companies continued to be promoted in the subsequent two years (FY20 and FY21), since most of the registrations took place during the Covid-19 pandemic from period March 2020 – March 2021 (Neti and Govil 2022)

Himachal Pradesh has seen the evolution and adaptation of FPCs, building on existing cooperative structures. The concept of collective action among farmers in the state dates back to the pre-independence era, addressing issues of fragmented landholdings and limited market access. While FPCs in Himachal Pradesh are relatively new, their development since the mid-2010s indicates growing adoption. The state's Prakritik Khetti Khushhal Kisan Yojana promotes climate-resilient natural farming, an agricultural approach that avoids synthetic inputs and aligns with natural ecological processes. Several initiatives, including the HP Horticulture Development Project (World Bank-funded), National Bank for Agriculture and Rural Development (NABARD), Small Farmers Agribusiness Consortium (SFAC), State Project Implementing Unit (SPIU), and Dr. YSP University of Horticulture and Forestry in Solan, support natural farming in Himachal Pradesh. Companies like Chaupal Natural Farmer Producer Company, Solan Natural Farmer Producer Company, Pachhad Natural Farmers Producer Company Limited, Karsog Valley Farmer Producer Company Limited, and Kutlehar Naturals Farmer Producer Company Limited offer training, technical support and capacity-building programs. These initiatives have significantly promoted the sustainability and growth of FPCs in the state, indicating a promising future for farmer-driven collective enterprises in Himachal Pradesh.

## MATERIALS AND METHODS

Himachal Pradesh, a Himalayan state in northern India, which is situated between 30° 22' N to 33° 13' N latitude and 75° 23' to 79° 4' East longitude is known for its diverse agriculture and horticulture practices. In recent years, there has been growing interest in promoting sustainable agricultural practices; especially natural farming (NF). The secondary data has been collected from the Horticulture Development Project (funded by World Bank), National Bank for Agriculture and Rural Development (NABARD), Shimla and Small Farmers Agribusiness Consortium (SFAC). There are total 216 registered farmer producer companies in Himachal Pradesh according to the secondary data collected from Horticulture Development Project (funded by World Bank), National Bank for Agriculture and Rural Development (NABARD) and Small Farmers Agribusiness Consortium (SFAC). The highest number of FPCs registered is in Shimla district (36) followed by Mandi (33), Sirmaur (31) and Kangra (26). These four district accounts more than 50 per cent of total FPCs registered in Himachal Pradesh.

**Linear Growth Rate :** The trends in the growth of FPCs in the Himachal Pradesh were estimated using linear growth rates (LGR).

$$Y = a + bt$$

where,

Y = Number of FPCs registered (2015-2023)

t = Time variable in year (1, 2, .....9)

a = Constant

b = Rate of change

The linear growth rate was calculated as:

$$\text{Linear growth rate} = b/y \times 100$$

where, b = regression coefficient, Y= Mean value of Number of FPCs registered (2015-2023)

**Autoregressive Integrated Moving Average (ARIMA) Model:** Autoregressive Integrated Moving Average (ARIMA) model was used for forecasting the growth of FPCs in the state of Himachal Pradesh with the help of R-studio software. The "R"-based software packages "tseries" and "forecast" were used to carry out the trend series analysis and forecasting of the growth of FPCs in Himachal Pradesh from 2024-2030. It forecast seven year (2024-2030) registered FPCs using forecast, prophet library and validation. Time series data are organized by time order and applied to develop and improve forecasting models. Prophet can handle trend shifts and outliers. The ARIMA model depends upon error lags *i.e.*, the difference between forecasted and actual outcomes. For the ARIMA model, data must be check for stationary autocorrelation. The Auto Arima function will check via autocorrelation function (ACF) and Partial autocorrelation function (PACF). Once the model is fitted

using the Akaike Information Criteria (AIC), the best-fitted model is selected for forecasting. If the selected model does not meet the criteria, the process is repeated. Finally, the statistical validity of the prediction is verified using the Box-Ljung test (Thapa 2022)

## RESULTS AND DISCUSSION

**Status of Farmers' Producer Companies (FPCs) in Himachal Pradesh:** The Linear Growth Rate (LGR %) was evaluated as 35.11 per cent indicates that since the year 2015 every year the number of registration has grown at the rate of 35 per cent. It is due to the policy support provided from state government along with support and assistance extended through Small Farmers Agribusiness Consortium (SFAC), National Bank for Agriculture and Rural Development (NABARD) as well as countless efforts of Horticulture Development Project (funded by World Bank) working at the grass root level. 53 per cent of Producer Companies are working with Horticultural crops and 41 per cent are dealing with agricultural and allied activities, while only very few are active in the areas of handicrafts, dairy, fishery and forestry).

**Forecasting the growth of Farmer Producer Companies (FPCs) in Himachal Pradesh:** There is blue line through which only one spike of black line are crossing which means that data is not highly correlated with itself and this pattern show that data is stationary (Fig. 3). The partial correlation function (PACF) will be employed for checking the stationary of the data, PACF has no issue because there are minimum spikes and none of them are crossing blue lines.

The last stationary test is by augmented dicky-fuller test (ADF) where the p value of ADF should be less than 0.5 or at least it should be 0.5. If the value p value is less than or equal to 0.5 then it means data is not stationary and estimated p value is 0.57 which is means data is stationary. Now comes AutoArriva function which shows the best fitted model comes in the ARIMA (p, d, q) and the best model is (0, 1, 0) where the Akaike information criterion (AIC) value should be as small as possible. The forecasting of the data for the next 7 years at 95 percent of confidence interval will be filtered (Fig. 4). The last step is to validate the test with the help of Box-Ljung test; this test should have p value more than 0.5 which means data is not autocorrelated (Table 2). There will be highest 605 more companies and lowest will be 182 more companies listed in the state in next seven years at 95 per cent of confidence interval. The Auto-Regressive Integrated Moving Average (ARIMA) show there is upward growth from the year 2020-2023 (Fig. 4). If the company sustains and work effectively for a long term it will enhance the human productivity and therefore sustainability of company will be influenced. Small and marginal farmer's livelihood sustainability to a great

extent will depend upon the equitable access to product markets.

**SDGs Relevant with Farmer Producer Companies (FPCs):** Agriculture can positively help in achieving seven SDGs (Nicholls et al. 2020) and five of these were the same as observed in study by Nhemachena et al. (2018), namely SDGs 1, 2, 12, 13 and 15. The others are as follows: SDG3-ensure healthy lives and promote wellbeing and SDG8-promote sustained, inclusive and sustainable economic growth. Based on the SDGs and the role of FPCs for furthering the same, developed the framework (Table 3), briefly describing SDGs related to agriculture that can be impacted by FPCs. FPCs are considered to be beneficial to

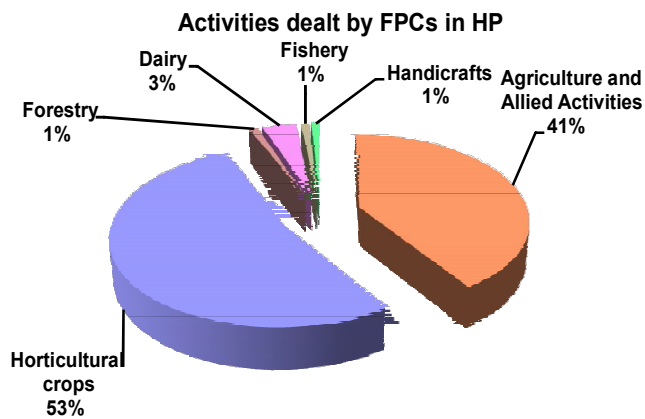


Fig. 2. Various activities dealt by FPCs in Himachal Pradesh

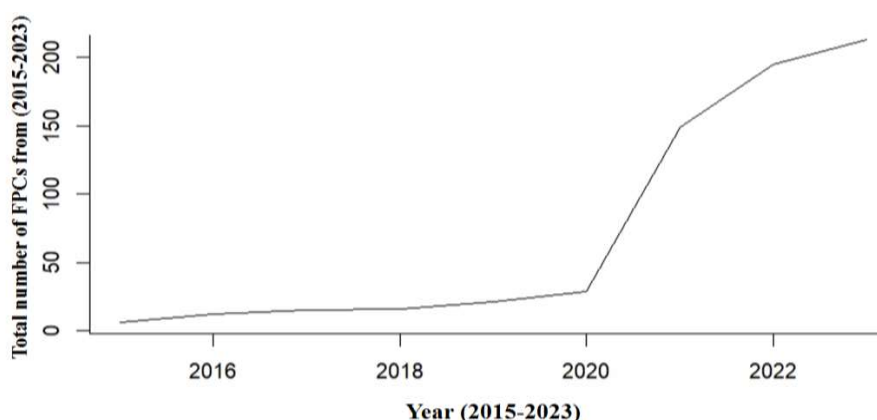


Fig. 1. Trend analysis of FPCs in Himachal Pradesh

Table 1. District wise number of FPCs registered in Himachal Pradesh (2015-2023)

District	Number of FPCs Registered (2015-2023)									
	2015	2016	2017	2018	2019	2020	2021	2022	2023	
Bilaspur	-	-	1	1	-	-	5	4	1	
Chamba	-	-	-	-	-	-	16	4	1	
Hamirpur	-	-	-	-	1	-	3	2	1	
Kangra	1	-	1	-	-	2	10	6	6	
Kinnaur	-	-	-	-	-	2	6	1	2	
Kullu	-	-	-	-	-	-	12	2	1	
Lahul and Spiti	-	-	-	-	-	-	-	2	1	
Mandi	1	-	1	-	-	1	14	15	1	
Shimla	1	2	-	-	4	-	20	5	4	
Sirmaur	-	2	-	-	-	1	26	1	1	
Solan	1	2	-	-	-	-	3	2	-	
Una	2	-	0	-	-	2	5	2	2	
Total FPCs registered	6	6	3	1	5	8	120	46	21	
Cumulative	6	12	15	16	21	29	149	195	216	
Total Paid up capital (₹ crore)	21.11	7.49	375.87	6.75	18.30	239.89	273.12	65.27	51.98	
Cumulative	21.11	28.60	404.47	411.22	429.52	669.41	942.53	1007.80	1059.78	
Current annual growth rate (%)					35.11					

the individual farmers owing to the short-term and long-term financial benefits (direct) and in terms of services and reduced costs (indirect benefits). These do grip the promise of realising no poverty (Goal 1) and reduce inequality by increasing income of poor (Goal 10). FPCs contribute towards better health and nutrition and sustainable agriculture (Goal 2) due to organic farming practices is well accepted (Mourya and Mehta 2021).

FPCs play a crucial role in making food accessible for poor where accessibility of food is very much important to achieve the Sustainable Development Goals (SDGs-2) in developing countries like India (Trebbin and Hassler 2012). Poverty is another issue resolved by FPCs as less amount of equity capital is accumulated during the initial phase of business. The strategy emerged from studies in Asia and Africa for contravening such vicious cycles of poverty was to harmonize small agro-enterprise. Accompanying with complementary interventions such as positive spill-overs including technological innovation, rural credit systems, communications, human capital formation and physical infrastructure (Singh 2012).

**Case study of natural farmer producer companies in Himachal Pradesh:** The consolidated information that brings together all the details of the natural farmer producer companies in Himachal Pradesh is given in Table 4.

#### Trend Analysis

**POPI support:** Most of these companies are receiving technical support from Dr. YS Parmar University of Horticulture and Forestry, Nauni, which indicates the importance of institutional support in establishing and running these companies.

**Diverse business activities:** The business activities range from processed items, spices, cereals, vegetables, fruits,

pulses, garlic, and ginger. This diversification helps in risk mitigation and ensures a steady income for the farmers.

**Member participation:** The number of members varies significantly among the companies, with Pacchad having the highest at 112 members, and Kotlehar the lowest at 0 members. This could reflect the varying levels of community engagement and trust in these initiatives.

**Equity mobilisation:** There is a noticeable range in equity mobilized, with Pacchad mobilizing the highest amount at ₹1.47 lakhs, indicating perhaps a greater level of investment and financial health, while Kotlehar has the lowest at ₹0.18 lakhs.

**Focus on value addition:** Companies like Chaupal and Solan are focusing on value addition and processing, which can significantly enhance the profitability and marketability of their products.

The trend suggests that institutional support, member engagement and diversification of activities are crucial factors for the success and sustainability of Natural Farmer Producer Companies in Himachal Pradesh

The Linear Growth Rate (LGR) of 35.11 percent indicates a significant increase in the number of Farmer Producer Companies (FPCs) registered in Himachal Pradesh from 2015 to 2023. This growth is attributed to policy support from the state government and assistance from institutions such as the Small Farmers Agribusiness Consortium (SFAC), National Bank for Agriculture and Rural Development (NABARD), and the World Bank-funded Horticulture Development Project. A majority (53%) of FPCs are engaged in horticultural crops, while 41% are involved in agricultural and allied activities. Only a few FPCs are active in areas like handicrafts, dairy, fishery, and forestry, indicating a potential area for diversification. Districts like Kangra, Mandi, Shimla, and Sirmaur have the highest number of FPC registrations, showcasing regional concentration. The total paid-up capital of FPCs has grown significantly, reaching ₹1059.78 crore by 2023, reflecting strong financial backing and investments. Using the ARIMA model, the forecast indicates a continuous upward trend, with the number of FPCs expected to reach between 394 and 605 by 2030. This growth will enhance human productivity and sustainability of FPCs, ultimately benefiting small and marginal farmers by providing better market access. FPCs contribute to achieving several Sustainable Development Goals (SDGs) including No Poverty (Goal 1), Zero Hunger (Goal 2), Gender Equality (Goal 5), Decent Work and Economic Growth (Goal 8), Reduced Inequalities (Goal 10), Sustainable Consumption and Production (Goal 12), Climate Action (Goal 13), and Peace, Justice, and Strong Institutions (Goal 16). FPCs play a critical role in improving the livelihood of smallholder

**Table 2.** Farmers' producer companies growth forecast of Himachal Pradesh

Year	Point forecast	Lo 95	Hi 95
2024	238.875	159.0239	318.7261
2025	264.750	151.8234	377.6766
2026	290.625	152.3188	428.9312
2027	316.500	156.7977	476.2023
2028	342.375	163.8224	520.9276
2029	368.250	172.6555	563.8445
2030	394.125	182.8587	605.3913

Best model: ARIMA(0,1,0) with drift

Augmented Dickey-Fuller Test

Dickey-Fuller = -1.999, Lag order = 2, p-value = 0.5728

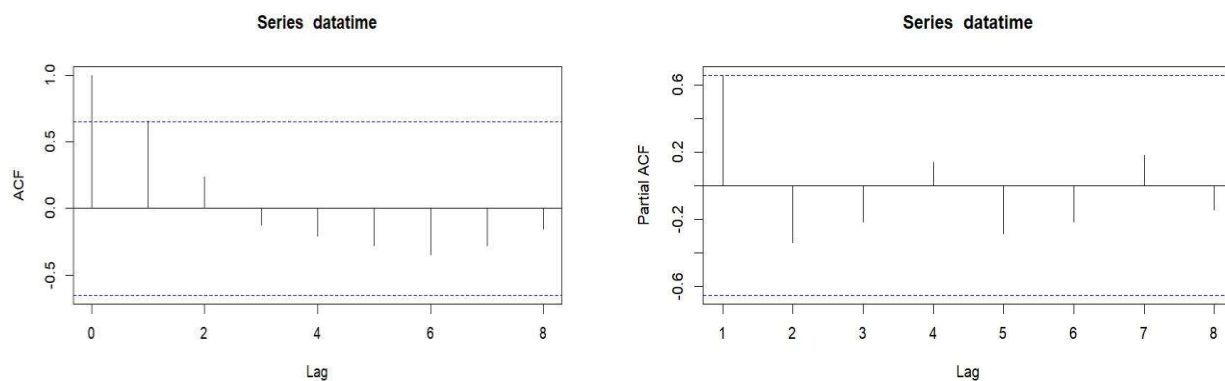
Box-Ljung test

X-squared = 0.64048, df = 2, p-value = 0.726

farmers, promoting sustainable farming practices, and fostering inclusive economic growth.

To ensure the sustainability and growth of Farmer Producer Companies (FPCs), several recommendations should be considered. Firstly, it is essential to encourage FPCs to diversify into areas such as handicrafts, dairy, fishery, and forestry to reduce dependency on a single sector and increase income sources. Promoting value addition and processing activities can enhance the profitability and

marketability of products. Implementing comprehensive training programs focused on sustainable farming practices, advanced marketing techniques, and financial management is crucial. Continuous support and capacity-building initiatives will improve the skills and knowledge of FPC members. Strengthening institutional support by fostering stronger collaboration between government institutions, NGOs, and educational institutions can create a holistic and supportive ecosystem for FPCs. Facilitating knowledge



**Fig. 3.** Plot of autocorrelation function and partial autocorrelation function of residuals for farmer producer companies (FPCs)

**Table 3.** Sustainable development goals (SDGs) related with farmer producer companies (FPCs)

SDGs	Targets
Goal 1: No poverty	FPCs can lift smallholder farmers out of poverty by improving their farm incomes, access to inputs and credit at lower costs. Providing better market access and bargaining power for farmers and reducing post-harvest losses through better storage and processing facilities (Papnai et al., 2021)
Goal 2: Zero hunger	FPCs improve food security and nutrition by promoting sustainable farming practices that increase productivity and reducing food waste through better storage and processing techniques. Linking farmers to markets where they can sell their produce at fair prices (Mourya and Mehta 2021)
Goal 5: Gender equality	FPCs can empower women farmers by providing them with training and capacity building programs. Encouraging their participation in decision-making processes and creating new income-generating opportunities for women
Goal 8: Decent work and economic growth	FPCs can create new jobs in rural areas by promoting agro-processing and value addition activities instead of selling raw in the market. Providing farmers with better market access, which can lead to increased demand for labour and encouraging entrepreneurship among farmers
Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialisation and foster innovation	FPCs increase the access of small-scale industrial and other enterprises particular in developing countries like India Provide financial services to small scale farmer in order to build infrastructure including affordable credit and their integration into value chains and markets (Mourya and Mehta 2021)
Goal 10: Reduced inequalities	FPCs can help reduce inequalities between small and large farmers by providing small farmers with access to the same resources and services as large farmers. Giving them a stronger voice in the market and helping them to improve their livelihoods (Venkatesan et al., 2020)
Goal 12: Ensure sustainable consumption and production patterns	FPCs can promote sustainable consumption and production patterns agriculture by encouraging farmers to adopt environmentally friendly practices. Helping farmers to improve the efficiency of their resource use that reduce food wastages.
Goal 13: Climate action	FPCs can help in mitigating climate change by promoting practices that sequester carbon in the soil and reduce greenhouse gas emissions from agriculture. It also helps farmers to adapt to the effects of climate change (Zobeidi et al., 2022).
Goal 16: Peace, justice and strong institutions	Develop effective, accountable and transparent institutions. Ensure responsive, inclusive, participatory and representative decision-making at all levels of institutions (Mourya and Mehta 2021)



## Forecasts from ARIMA(0,1,0) with drift

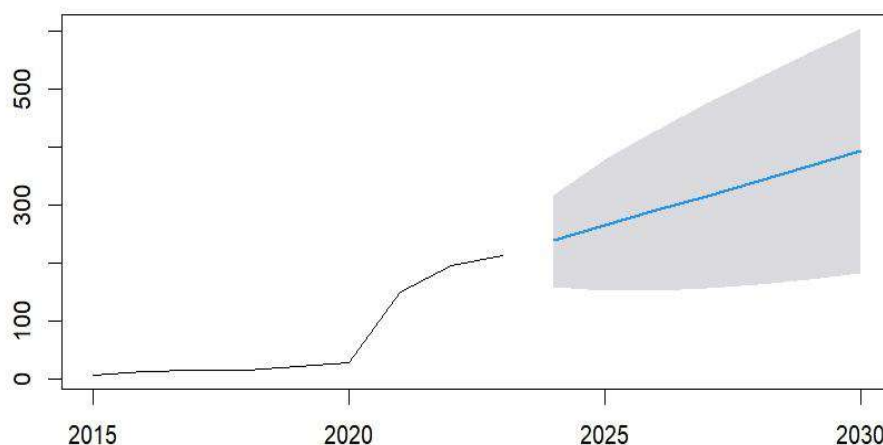


Fig. 4. Forecasting of farmer producer companies in Himachal Pradesh from (2024-2030) using ARIMA model

Table 4. Natural farmer producer companies in Himachal Pradesh

Company name	Major business activity	POPI name	Total members	Equity mobilised (₹ Lakhs)
Chaupal naturals farmers producers company ltd.	Processed items, apple, fruits & vegetables, pulses, millets	Dr. YS Parmar University of Horticulture and Forestry, Nauni	41	0.97
Solan natural farmer producer company ltd.	Spices, cereals (food grains & millets), vegetables, pulses	Dr. YS Parmar University of Horticulture and Forestry, Nauni	42	0.73
Pacchad natural farmers producers company ltd.	Garlic and ginger	Dr. YS Parmar University of Horticulture and Forestry, Nauni	112	1.47
Karsog naturals farmers producer company ltd.	Vegetable, millets, fruits, soyabean, coriander, wheat, gram, apple	Dr. YS Parmar University of Horticulture and Forestry, Nauni	10	0.35
Kutlehar naturals farmers producer company ltd	Growing of crops, market gardening, horticulture	Non-government company	0	0.18

sharing and best practices through workshops, seminars, and online platforms is also important. Increasing access to low-interest loans, grants, and subsidies will provide financial support for FPCs. Encouraging private sector investment and public-private partnerships can bring in additional resources and expertise. Finally, establishing a robust monitoring and evaluation framework to continuously assess the performance, impact, and challenges faced by FPCs is vital. Using data-driven insights to make necessary adjustments to policies and programs will ensure the sustainability and growth of FPCs.

### CONCLUSIONS

The significant increase in Farmer Producer Companies (FPCs) in Himachal Pradesh from 2015 to 2023, driven by state policy support and institutional assistance, has led to substantial financial growth and enhanced market access for small and marginal farmers. Most FPCs are engaged in horticultural and agricultural activities, but there's potential for diversification into other sectors. Forecasts suggest

continued growth, contributing to various Sustainable Development Goals. Recommendations for sustainability include diversification, value addition, training programs, stronger institutional support, financial assistance, private sector involvement, and robust monitoring frameworks. These efforts aim to improve smallholder farmers' livelihoods and foster inclusive economic growth.

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Received 12 October, 2024; Accepted 17 January, 2025



# Perceived Climate Change Risks and Constraints Faced by Dairy Farmers in Odisha

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**Abstract:** The study examines the key risks perceived by dairy farmers in Odisha and the challenges encountered when implementing climate adaptation strategies such as changing the micro-climate of the cattle shed. About 68% of dairy farmers were aware of climate-related risks in 2022 and struggled to adapt due to economic limitations and lack of access to resources and information. The binary logistic regression analysis indicates that perceived risks, such as animal infertility and disease incidence, significantly affect farmers' decisions to alter their cattle management practices. Conversely, factors like family size hinder their ability to make these changes. Additionally, the extent of adoption is limited by market dynamics, including milk prices and information asymmetry, as prioritized using Garrett's ranking. These barriers impede effective adaptation and emphasize the necessity for comprehensive support systems, including fair pricing for farmers, climate education, enhanced access to information, and awareness campaigns focused on climate change, especially concerning dairy animals.

**Keywords:** Dairy farmers, Change in microclimate, Disease incidence, Perceived risk

Globally, climate change affects many aspects of human life, society and the natural environment. The impact of climate change has a serious consequence on many sectors of the economy and its ill effects are particularly pronounced in developing countries like India (Rao et al., 2016, Das et al., 2022). There is growing evidence that persons depending on climate-sensitive agriculture and allied sectors would suffer economically due to climate change (Ravindranath 2011, Madhi et al., 2021, Singh and Jatav 2023). The sixth assessment report of the Intergovernmental Panel on Climate Change (IPCC) predicted that it is inevitable to decrease the earth's temperature below 1.5° C in any scenario (IPCC AR6 2021). The temperature rise will intensify the climate of extreme events such as cyclones, drought, floods, etc. In this respect, adopting various climate resilient practices becomes more crucial (Acharya et al., 2022, Shanabhoga et al., 2023, Shivakumarappa et al., 2023).

The global warming effect harms the production and productivity of crops and animals (Sirohi and Michaelowa 2007). The incidence of heat stress in dairy animals seriously affects their reproductive and productive performance (Sere et al., 2008). This results in a reduction in both the quality and quantity of milk. In addition to this, an increase in temperature increases the risk of metabolic disorders, lameness, mastitis, and other animal health issues (Sinha et al., 2017). All of these lead to a loss in income due to a decrease in milk quantity and quality, an increase in expenditures due to

repeat breeding, an increase in treatment costs, and possibly the death of animals due to a catastrophic incidence (Upadhyay et al., 2009). It necessitated continuous health monitoring of dairy animals. Appropriate adaptation and mitigation techniques may significantly reduce the climate change effect and help farmers sustain productivity during extreme climate changes. The adoption of adaptation techniques to climate change depends on four factors, i.e., (i) how farmers assess the climate change risk that poses in dairying, (ii) the financial position of the farmers, (iii) market assurance in terms of price realization, and (iv) appropriate information on climate, market and production aspects (Anoop et al., 2022).

Dairy farming is an integral part of livelihood in rural Odisha. Over 85 percent of the cattle population in Odisha is owned by landless, marginal, and small farmers who are vulnerable to climate extreme events (Odisha Economic Survey 2021-22). Erratic rainfall, flash floods during the rainy season, heat waves in summer, drought and dry periods every two years, notably in western Odisha, extreme coastal flooding, and cyclones are the main climate-induced dangers in Odisha. Climate change is likely to increase both the frequency and severity of such disasters in the future. The extreme climate occurrences cyclones and flooding are wreaking havoc on Odisha and the majority of districts impacted by both disasters. The super cyclone of 1999 on the Odisha coast killed 55000 cows (CSO 2000) and several such instances have been observed in cyclones like Phailin

(2013), Fani (2019), Amphan (2020). In light of the significance of dairy to small and marginal farmers, determining the perceived effects of climate change on various aspects of dairy farming and constraints in adaptation will aid in the formulation of adaptation policies for future challenges (Kant et al., 2015, Singh and Singh 2023). In the milieu, the study captures perceptions regarding the effect of climate change on dairy animals and various constraints faced by dairy farmers.

## MATERIAL AND METHODS

The state of Odisha was chosen for the current study. There are 10 agro climatic zones in Odisha and each of the selected district belongs to a distinct agro climatic zone. Agro-climatic zones greatly influence dairy farmers' productivity and resilience by shaping factors such as fodder availability, livestock breeds, housing, management practices, and climate adaptation strategies. Primary data was collected using stratified random sampling. Six different districts (Balasore, Bargarh, Dhenkanal, Ganjam, Jagatsinghpur, and Sundergarh) and two blocks from each selected district were considered for primary data collection and two blocks from each selected district were considered for primary data collection (Table 1). The 360 dairy households (with strict inclusion criteria of at least three milch animals) were randomly selected from a cluster of villages. The information on perception regarding various climate aspects was collected following a well-structured interview schedule.

In the summer along India's eastern coast, temperatures often reach 40°C, sometimes climbing above 45°C, with humidity levels as high as 80-90%. To combat heat stress in their livestock, dairy farmers change the microclimate of the

cattle shed by utilizing electric fans, foggers, sprinklers, straw bedding, and heat-insulating materials. Considering this a binary logistic regression model was used to analyze the influence of the perceived factors on the likelihood of farmers adapting their cattle sheds' microclimate. The dependent variable in this study was binary, indicating whether or not the farmer decided to implement changes in the microclimate of their cattle shed (1 = Yes, 0 = No). The logistic regression model is appropriate for cases where the outcome variable is binary. The probability of adopting a microclimate change ( $P(Y=1)$ ) is modelled as a function of the independent variables, using the logit function:

$$\text{logit}(P) = \log \frac{P(Y=1)}{1-P(Y=1)} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k$$

Where,

$P(Y=1)$  is the probability of the farmer making a change.

$\beta_0$  is the intercept

$\beta_1, \beta_2, \dots, \beta_k$  are the coefficients of the independent variables  $X_1, X_2, \dots, X_k$ .

The logistic regression model was estimated using STATA. Odds ratios were computed to interpret the influence of each independent variable on the likelihood of adaptation, with confidence intervals and p-values used to assess statistical significance.

**Identifying constraints in adaptation:** Garrett's ranking technique has been used to prioritize the constraints in the adoption of management strategies.

$$\text{Per cent position} = \frac{100 \times (R_{ij} - 0.5)}{N_j}$$

Where,  $R_{ij}$  is rank given for  $i^{\text{th}}$  factor by  $j^{\text{th}}$  individual

$N_j$  is the number of factors ranked by the  $j^{\text{th}}$  individual

The percent position of each rank was converted into scores. Finally, the mean scores for all the factors are arranged and ranked in descending order to identify the important constraints.

**Table 1.** GPS locations of the study areas

Districts	Blocks	Latitude	Longitude
Balasore	Baliapal	21.65	87.28
	Bahanaga	21.34	86.76
Jagatsinghpur	Jagatsinghpur	20.25	86.17
	Kujang	20.30	86.53
Ganjam	Digapahandi	19.38	84.56
	Rangeilunda	19.26	84.77
Dhenkanal	Dhenkanal	21.11	85.58
	Bhuban	20.89	85.83
Bargarh	Attabira	21.37	83.78
	Barapali	21.18	83.60
Sundergarh	Sundergarh	22.13	84.05
	Gurundia	21.85	84.78

## RESULTS AND DISCUSSION

**Socio-economic profile:** The socioeconomic profile of 360 farmers indicate average age of 47.36 years indicating most are in their mid-40s (Table 2). On average, farmers have completed secondary education with 10.4 years of dairy farming experience. The cattle sheds are predominantly semi-pucca to pucca types. The average herd size is 13.21, though most farmers are smallholders, typically owning 3-4 milch animals. The animals are mainly indigenous cows and crossbreeds, including Haryanvi and Jersey cows.

**Perceived risk due to climate change:** About 68 percent of the farmers were aware of the risk due to climate change while others were not aware of such issues due to illiteracy

(Table 3). The significant proportion of farmers (63.33%) expressed concerns about a reduction in both the quantity and quality of milk production, primarily attributing it to environmental factors. Furthermore, farmers also observed increased occurrences of animal infertility (61.11%) and heat stress in dairy animals (50.27%). The milk yield of animals negatively correlated with the temperature-humidity index (THI) (Behera et al., 2018). THI above critical level causes heat stress in dairy animals which also increases the chances of animal infertility. There was also a reduction in feed and fodder intake in dairy animals perceived by about 43 per cent of respondents. Heat stress induces behavioral and metabolic modifications, including decreased feed intake and metabolic activity, and consequently a decrease in productivity (Sirohi and Michaelowa 2007). About 47 per cent farmers expressed that there was more incidence of diseases due to after flood effects. During flood the water got contaminated due to deceased animals and the animals which forced to drink that contaminated water got affected. The extreme climatic events like flood and cyclone cause death of the animals and reduce availability of feed and fodder as perceived by 37 and 38 per cent respondents respectively.

The binomial logistic regression analysis examining how farmers' perceived risks influence their decisions to modify the microclimate of their cattle sheds (Table 4). Several factors significantly affect these decisions, including education, herd size, and access to climate information. Higher education levels and larger herds are associated with a greater likelihood of implementing changes, possibly due to a better understanding of climate impacts and resource management. Conversely, family size has a negative and significant effect, suggesting that larger families may face constraints in time or resources, making them less likely to adjust their practices. Among the perceived risks, the problem of animal infertility (coefficient = 0.19) and the incidence of diseases) are particularly influential, indicating that farmers are motivated to take action in response to these serious concerns. However, perceived risks related to reductions in milk quantity and quality, as well as the unavailability of feed and fodder, do not significantly influence decision-making. This discrepancy may reflect a lack of awareness or information regarding the long-term impacts of these risks. Overall, the model accounts for 50% of the variability in farmers' decisions (Pseudo  $R^2 = 0.50$ ) and is statistically significant (LR  $\chi^2 = 228.35$ ,  $n = 360$ ), underscoring the importance of these factors in shaping cattle management practices in response to climate risks.

Farmers in the study area face challenges in getting profitable prices for their milk due to the underdeveloped

**Table 2.** Socioeconomic profile of the farmers

Particulars	Average (n=360)
Age (years)	47.36 (7.37)
Education (0=illiterate, 1= primary, 2= secondary,3= graduation & above)	1.91 (0.77)
Experience in dairy farming (years)	10.40 (2.21)
Type of cattle shed (Kutch=0, Semi-pucca=1, pucca=2)	1.36 (1.34)
Herd size (number)	13.21 (3.96)

Figures in the parenthesis represent the standard deviation

**Table 3.** Farmers perceived risk due to climate change

Perceived risks	Frequency (N=360)
No awareness on climate change effects to dairy animals	248 (68.88)
Reduction in milk quantity and quality	228 (63.33)
Problem of animal infertility/repeat breeding	220 (61.11)
Incidence of heat stress in animal	181 (50.27)
Incidence of diseases become more	170 (47.22)
Reduction in feed and fodder intake by the animal	158 (43.88)
Unavailability of feed and fodder	138 (38.33)
Reduction in longevity of animal	134 (37.22)

Figures in the parenthesis represent the percentage of the total respondents

**Table 4.** Effect of farmers' perceived risk on farmers' decision to change in microclimate of the cattle shed

Variables	Coefficient	Standard Error
Age	0.0023	0.0024
Education	0.059**	0.024
Herd Size	0.017***	0.004
Experience	0.003	0.008
Sources of climate information	0.055***	0.01
Family size	-0.046***	0.013
Perceived Risks (Yes=1, No=0)		
Reduction in milk quantity and quality	0.02	0.05
Incidence of heat stress in animal	0.08	0.05
Problem of animal infertility	0.19***	0.04
Reduction in feed and fodder intake by the animal	0.05	0.039
Incidence of diseases	0.08**	0.038
Reduction in the longevity of animal	-0.01	0.039
Unavailability of feed and fodder	0.002	0.039

Pseudo  $R^2=0.50$ , LR  $\chi^2 = 228.35$ \*\*\*,  $n=360$

**Table 5.** Constraints faced by dairy farmers

Constraints	Overall	
	Garret score	Rank
Unsatisfactory milk prices	60.39	I
Lack in information	58.79	II
Paucity of money	54.46	III
Increase in prices of feed and fodder	54.31	IV
No Training	49.83	V
Non-availability of critical inputs	46.71	VI
Non-availability of credit	43.20	VII
Non-availability of labor	32.33	VIII

dairy market, as mentioned by Kale et al. (2016) (Table 5). Low milk yields are common, partly due to the prevalence of non-descriptive indigenous cattle. Rising feed and fodder prices by further reduce productivity (Acharya et al., 2022). About 65.27% of farmers belong to cooperative societies, benefiting from subsidized feed, while non-members struggle with rising concentrate costs. Many farmers rely on dry fodder and concentrate, with little cultivation of green fodder in the area. Irregular milk payments and poor bargaining power are also issues, alongside a lack of information and financial resources. To enhance climate adaptation, training on insurance policies and credit facilities is needed, with key barriers including low milk prices, poor animal productivity, and limited awareness among farmers.

### CONCLUSIONS

The study provides valuable insights into the perceived risks of climate change and constraint among dairy farmers. A significant proportion (68%) were aware of climate-related risks, yet many face challenges in adapting to these threats, primarily due to economic constraints and limited access to resources and information. The analysis indicates that specific perceived risks—particularly animal infertility and increased disease incidence—significantly influence farmers' decisions to modify the micro climate of the cattle shed. However, factors such as family size negatively affect their ability to implement these changes. Despite the awareness of potential reductions in milk quantity and quality did not significantly motivate action, possibly indicating lack of understanding of the long-term impacts of these risks. Farmers also face considerable constraints while adapting which include unsatisfactory milk prices, lack of information, and rising feed costs. These barriers hinder effective adaptation and highlight the need for comprehensive support systems. Therefore, policy measures should be directed towards assuring remunerative prices to the farmers, educational programs on climate risks, improving access to

climate information, and creating awareness camps about climate change and its consequences, especially for dairy animals.

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Received 13 October, 2024; Accepted 24 January, 2025



# Influence of *Prosopis juliflora* on Breeding Success of Black-headed Ibis *Threskiornis melanocephalus*

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**Abstract:** The Black-headed Ibis (*Threskiornis melanocephalus*, Latham, 1790) is a large, white-water bird. Nehru Talai of Bhilwara district (Rajasthan, India) is an island-like structure with some exotic *Prosopis juliflora* (Vilayati babul) and native *Vachellia nilotica* (Babul), *Ziziphus mauritiana*, and *Salvedora persica* vegetations of which stands on the central island and provides an excellent nesting site of Black-headed Ibis. Bird census technique like the focal method was used to monitor Black-headed Ibis. Various nest parameters were examined from June 2023 to November 2023 at Nehru Talai. *Prosopis juliflora* is one of the top hundred recognized invasive species worldwide. The percentage of dropped eggs and nestlings per nest was higher in *Prosopis juliflora* than in *Vachellia nilotica* due to the branching angle of *Prosopis juliflora* being higher than *Vachellia nilotica*. In this article, we observed the impact of *Prosopis juliflora* on the breeding success of Black-headed Ibis and to conserve the near threatened Black-headed Ibis, the regular removal of *Prosopis juliflora* from the breeding sites.

**Keywords:** Black-headed Ibis, Nehru Talai, *Prosopis juliflora*, *Threskiornis melanocephalus*

The Black-headed Ibis (*Threskiornis melanocephalus*, Latham, 1790) is a large (65-75 cm long), wading, nomadic, and white-water bird. It has a long, downward-curving black bill and a prominently exposed black head and neck. The species is included in the Near Threatened (NT) category by IUCN (Bird Life International 2017) and is widespread resident throughout India (Grimmett et al 2011) and has a wide global range extending from Pakistan to Myanmar and sporadically to China and Japan (Ali and Ripley 1987). The nest of the Black-headed Ibis is a platform made of twigs and sticks that are covered in grass and threads and built on top of *Acacia*, *Prosopis*, and different types of *Ficus* in wetlands and nearby wetlands (Senma and Acharya 2010). The most preferred nesting tree species were "Vilayati babul *Prosopis juliflora*" and "Babul *Vachellia nilotica*" and Cattle Egrets *Bubulcus ibis* (Choudhary and Koli 2018). The influence of exotic plant invasions on the structure and functional attributes of native ecosystems has been extensively documented and debated (Wardle 2011). The complex interactions of invasive species with native ecosystems make invasion ecology an interesting and important area of research. *Prosopis juliflora*, a member of the *Fabaceae* family, is native to South and Central America. It was introduced to India to help recover degraded areas and provide fuel wood for the rural poor. Now it has become an aggressive weed in several parts of the country and poses a serious threat to native biodiversity (Mwangi et al 2005). Bird species and their habitats are declining worldwide (Birdlife International 2017) due to various threats, viz. habitat fragmentation, climate change and higher nest predation

(Robinson et al., 1995). Changing ecological conditions across the globe are creating new threats to birds and identifying these emerging threats will help design suitable strategies to conserve them. Negative correlation between *Prosopis juliflora* density and bird diversity has also been reported through a study on the relationship between tree species and avifaunal diversity (Khera et al., 2009). The higher number of fallen eggs and nestlings under *Prosopis juliflora* while the higher number of fledglings per nest on *Vachellia nilotica* relates the difference to the plant architecture (Chandrasekaran et al 2014). *Prosopis* and *Capparis* trees were the least diverse habitat for avian species (Koladiya et al., 2014). In contrast to native vegetation where a variety of food sources are available, *Prosopis* invaded areas were avoided by not only birds but also butterflies (Choudhary and Chishty 2020).

## MATERIAL AND METHODS:

**Study area:** For the study of the influence of *Prosopis juliflora*, Nehru Talai (25°21'26"N 74°38'19"E) of Bhilwara district (Rajasthan, India) was selected. There is an island-like structure with some vegetation of *Vachellia nilotica*, *Prosopis juliflora*, *Ziziphus mauritiana*, and *Salvedora persica* which stands on the central island and provides an excellent nesting site for Black-headed Ibis (Fig. 1).

**Methods:** The bird census technique like the focal method was used to monitor Black-headed Ibis (Colin et al 1993). Photographs were captured (NIKON P900) of the nest locations in *Prosopis juliflora* and *Vachellia nilotica*. The number of nests per tree, eggs per nest, number of fallen



eggs and nestlings, and final population at the fledgling stage were recorded in *Prosopis juliflora* and *Vachellia nilotica* for one breeding season (June 2023 to November 2023). The number of nests per tree was counted with the help of binocular (VANGUARD FR-1650W). We followed the all guidelines for conducting research on the nesting biology of birds (Barve et al., 2020). The percentage of dropped eggs and nestlings per nest was calculated.

Per cent of dropped eggs and nestlings per nest=

$$\frac{\text{Number of fallen eggs/nestlings per tree}}{\text{Number of eggs per tree}} \times 100$$

## RESULTS AND DISCUSSION

The Black-headed Ibis built 'platform nests', which consisted of an irregularly placed, loose assemblage of twigs and sticks. The major portion of the nesting materials belonged to the tree on which the nest was located. Vegetations like native trees, Babul *Vachellia nilotica*, Miswak *Salvedora percica*, and exotic tree Vilayati babul *Prosopis juliflora* were dominant in this wetland. On *Vachellia nilotica*, nests were mostly located at nodes with more than two branches (Fig. 2). Nests were distributed at mid-canopy

with mostly one nest per node (Fig. 3, Table 1). The nests in *Prosopis juliflora* were distributed at the upper canopy with all nodes and branches (Fig. 4). The number of nests per tree was higher in *Prosopis juliflora* (07) compared to *Vachellia nilotica* (06). There is no difference in the number of eggs per nest between *Prosopis juliflora* (03) and *Vachellia nilotica* (03). The number of fallen eggs (Fig. 5) and nestlings (Fig. 6) was higher under *Prosopis juliflora* (1.2) than *Vachellia nilotica* (0.3). The percentage of dropped eggs and nestlings per nest was 40 and 10 for *Prosopis juliflora* and *Vachellia nilotica*, respectively (Table 2). The number of fledglings per nest was higher in *Vachellia nilotica* (2.7) when compared to *Prosopis juliflora* (1.8). There were four or more individuals (including parents) per nest in *Vachellia nilotica* but only three individuals were recorded in *Prosopis juliflora* at the end of the reproductive season. The number of fledglings were higher in *Prosopis juliflora* when compared to *Vachellia nilotica* (Fig. 7 and 8).

## CONCLUSIONS

The results of this study unequivocally demonstrate that the *Prosopis juliflora* invasive tree is a serious threat to Black-headed Ibis nesting success. The branching pattern may be involved in the low nesting success in *Prosopis juliflora*. In *Vachellia nilotica*, the branching angle ranges from 20° to 100°, whereas in *Prosopis juliflora*, it primarily ranges from 80° to 180°. This could cause more eggs and nestlings in *Prosopis juliflora* to slide out of their nests. Evidence of the detrimental effects of this alien tree may be seen in the variation in the Black-headed Ibis population structure in *Prosopis juliflora* and *Vachellia nilotica* after the breeding season. *Prosopis juliflora* branching architecture overlaps and reaches in several directions, which may be the cause of the species' highest mortality. *Prosopis juliflora* has overtaken almost all of the wetlands in the state of Rajasthan.



Fig. 1. Nehru Talai, Bhilwara (Rajasthan, India)

Table 1. Nest site characteristics of Black-headed Ibis at Nehru Talai, Bhilwara (Rajasthan, India)

Nesting trees	NT	TH (M)	UNH (M)	LNH (M)	GBH (M)	Nesting canopy	DTW (M)	Number of nests
<i>Vachellia nilotica</i>	04	8	5	4.5	0.5	Mid canopy	7	27
<i>Prosopis juliflora</i>	12	2	2	0.3	0.15	Upper canopy	1	85
<i>Salvedora percica</i>	02	6	4	3.5	3.0	Upper canopy	2	14

NT- Number of trees, TH- Tree height, UNH- Uppermost nest height, LNH- Lowermost nest height, GBH- Girth at breast height, DTW- Distance to water

Table 2. Comparison of nests of Black-headed Ibis on *Prosopis juliflora* and *Vachellia nilotica* at Nehru Talai, Bhilwara (Rajasthan, India)

Tree	Number of nests per tree	Number of eggs per nest	Number of dropped eggs/nestlings per nest	Number of fledglings per nest	Percentage of dropped eggs per nest
<i>Prosopis juliflora</i>	07	03	1.2	1.8	40
<i>Vachellia nilotica</i>	06	03	0.3	2.7	10



**Fig. 2.** Black-headed Ibis nests on *Vachellia nilotica*



**Fig. 3.** Black-headed Ibis nests on *Prosopis juliflora*



**Fig. 4.** Black-headed Ibis nests on all branches of *Prosopis juliflora*



**Fig. 5.** Fallen egg of Black-headed Ibis from *Prosopis juliflora*



**Fig. 6.** Fallen nestling of Black-headed Ibis from *Prosopis juliflora*



**Fig. 7.** Fledglings on *Vachellia nilotica*



**Fig. 8.** Fledglings on *Prosopis juliflora*

Therefore, to prevent further loss of this near-threatened bird species, *Prosopis juliflora* must be routinely mechanically removed before Black-headed Ibis arrive at nesting locations.

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Received 30 September, 2024; Accepted 24 January, 2025



# Seasonal Variations in Diet Choice of the Indian Flying Fox, *Pteropus medius*

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**Abstract:** The seasonal diet choice of the Indian flying fox, *Pteropus medius*, was carried out in Lucknow, Uttar Pradesh, India, from March 2023 to February 2024. A total of 885 fecal samples (bolus and guano) were gathered from nine roost sites and analyzed. The analysis indicated that the diet predominantly consisted of six genera across five plant families, with *F. racemosa* and the Moraceae family being the most prevalent. Seasonal variations in dietary composition included the consumption of *M. alba*, *P. guajava*, and *N. cadamba*, while *F. racemosa* and *F. benjamina* which were abundant during the summer, monsoon, and post monsoon periods. Generalized Linear Model (GLM) utilizing a gamma distribution demonstrated that *P. guajava* and *F. racemosa* were the most frequently consumed species during the monsoon and summer seasons in the guano. The significant seasonal effects with increased consumption was observed during the monsoon and summer. The bolus analysis indicated a preference for *F. benjamina* and *M. indica*. These findings highlight the seasonal dietary adaptations of *P. medius*, emphasizing the essential role of specific plant species, particularly during the monsoon and summer seasons, in fulfilling its nutritional requirements.

**Keywords:** *P. medius*, Indian flying fox, *Ficus*, Diet analysis

The Indian flying fox, *Pteropus medius* (Temminck 1825), is a significant species within the order Chiroptera and the suborder Yinpterochiroptera (Lei and Dong 2016). It ranks among the largest bat species globally. This species is endemic to South Asia and plays crucial ecological roles, including pollination, seed dispersal, and nutrient cycling, impacting over 114 plant species (Aziz et al., 2021). The Indian flying fox is essential for sustaining ecosystem functionality, as it substantially contributes to various ecological processes such as pollination, seed dispersal, and nutrient cycling (Goveas et al., 2006, Kumar and Elangovan, 2019, Madala et al., 2022).

Despite their critical ecological significance, bats face numerous threats, including hunting and the culling of individuals, which are frequently perceived as pest species (Epstein et al., 2009). On a global scale, *P. medius* is classified as "Least Concern" on the IUCN red list of threatened species (Tsang 2020) and is afforded protection under Schedule II of the wildlife (protection) amendment act, 2021 in India, as well as by the convention on international trade in endangered species of wild fauna and flora (CITES: Appendix II). Bats, such as the Indian flying fox, exhibit distinctive feeding behaviors that set them apart from other mammalian species. Numerous studies have examined the dietary habits of various *Pteropus* species; however there is a paucity of research focusing on their dietary patterns within specific geographical contexts. In the Indian subcontinent, only a

limited number of investigations have been conducted regarding the diet of *P. medius* (Mahmood-Ul-Hassan et al., 2010, Javid et al., 2016, Ashwin and Jayakumar 2019, Tiwari et al., 2019, Basharat et al., 2021). The dietary preferences of *P. medius* are known to fluctuate in accordance with the seasonal availability of fruits (Stier and Mildenstein 2005). The present study concentrates on the region of Lucknow, Uttar Pradesh, which is characterized by a rich diversity of angiospermic flora, encompassing more than 1,263 plant species across 705 genera and 140 families. This assemblage includes 989 species of dicotyledons and 274 species of monocotyledons. The representation of monocotyledons is comparatively lower, with significant contributions from the Poaceae and Cyperaceae families. Specifically, out of the 274 monocotyledon species, 176 are classified within these two families, while the remaining 98 species are distributed among 23 additional families (Singh et al., 2020). The objective of this study was to investigate the seasonal dietary preferences of *P. medius* in Lucknow, Uttar Pradesh. These insights aim to support effective conservation strategies and ensure the long-term survival of this ecologically significant species.

## MATERIAL AND METHODS

**Study area:** The present study was conducted at Lucknow, capital of Uttar Pradesh, is situated 123 meters above sea level. It is located between latitudes 26.30° and 27.10° North and longitudes 80.30° and 81.13° East.

**Study sites:** The nine roost sites of *P. medius* in Lucknow were selected for this study (Table 1). A cartographic representation of the study locations was created using QGIS software (version 3.36.0) (Fig. 1). According to the India meteorological department of Lucknow; the winter season spans December, January, and February, while March, April, and May constitute the summer season. The monsoon season occurs in June, July, August, and September. The post monsoon season includes October and November.

**Field surveys and sample collections:** Field surveys were conducted to gather fecal pellets from Indian flying foxes between March 2023 and February 2024. Sample collection was done during the morning hours, specifically from 8 AM to

12 PM, when the flying foxes were observed at their roosting locations. It has been documented that Indian flying foxes consume soft fruits, extracting juice and subsequently expelling the remnants, referred to as bolus, while the material excreted from the gastrointestinal tract is termed guano (Goveas et al., 2006). Fresh bolus and guano from *P. medius* were collected by placing old plastic sheets on the ground of diurnal roosting sites (Hodgkison et al., 2003). The samples were randomly selected from the overall population of flying foxes, following the methodology established by Parry-Jones and Augée (2001). The collected samples were then securely contained in sealed receptacles and transported to the laboratory for further analysis.

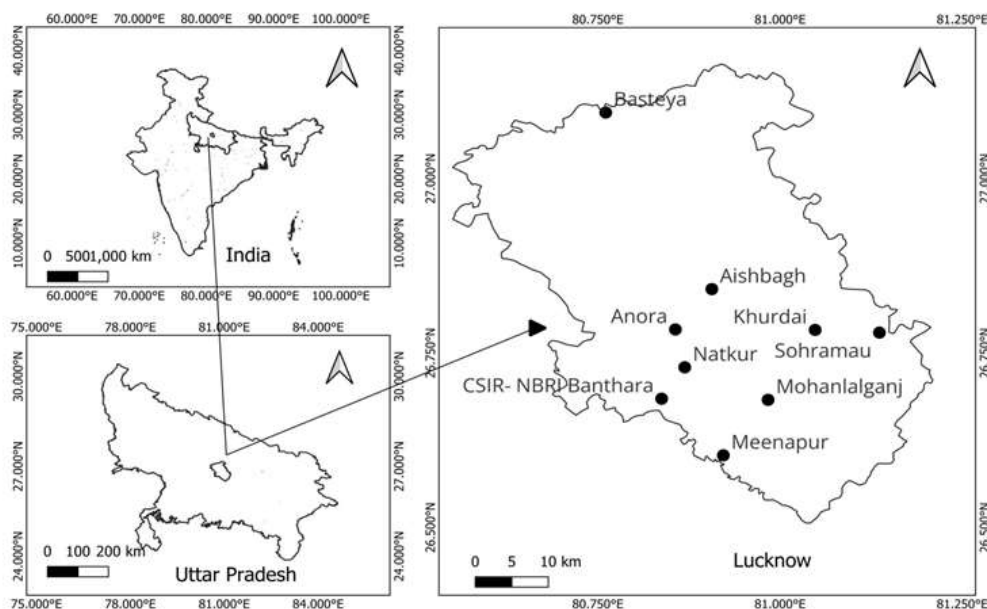
**Diet analysis and species recognition:** The samples were transferred into a 100 ml glass container, to which 50 ml of distilled water was subsequently added. The resulting mixture was agitated thoroughly to facilitate the separation of undigested food particles. The seeds and other components that were extricated from the bolus and guano were then enumerated and visually identified through comparison with reference seeds. This identification process was conducted utilizing a handheld magnifying glass and a stereomicroscope (RADICAL RSMr-3), in accordance with the methodology outlined by Hodgkison et al. (2003).

**Data analysis:** Generalized Linear Model (GLM) incorporating a gamma distribution and log link function was employed to examine the relationship between the frequency of plant species found in guano/bolus and seasonal variations. The dependent variable in this analysis was

**Table 1.** Geo-coordinates and elevations of roost sites of the *Pteropus medius*

Site name	Latitude (DD)	Longitude (DD)	Elevation (m)
Anora	26.7839	80.8564	145.58
Aishbagh	26.8417	80.9064	148.48
Basteya	27.0947	80.7608	140.33
CSIR- NBRI Banthara	26.6847	80.8375	146.3
Khurdai	26.7832	81.0481	142.64
Meenapur	26.6036	80.9222	147.63
Mohanlalganj	26.6831	80.9836	142.84
Natkur	26.7297	80.8692	145.34
Sohramau	26.7794	81.1364	142.8

(DD = Decimal Degree, m = Meter)



**Fig. 1.** Study area map of *Pteropus medius*

species frequency, while the independent variables included plant species and seasonal categories (winter, summer, monsoon and post-monsoon). The adequacy of the model fit was assessed through the deviance/df and Pearson chi-square/df ratios, with values approaching 1 indicating satisfactory fit. The Likelihood ratio Chi-Square test was utilized to evaluate improvements in the model, whereas the Wald Chi-Square test was used to analyze the effects of species and seasons. Parameter estimates B values (the regression coefficients from the GLM analysis, indicating the relative influence of each plant species on dietary frequency) and estimated marginal means were computed. All statistical analyses were conducted using SPSS (version 21). The data were expressed as frequency of occurrence for each plant species to determine the food preferences of *P. medius* (Mahmood-UI-Hassan et al., 2010).

$$\text{Frequency of occurrence} = \frac{\text{Number of samples in which particular plant source occurred}}{\text{Total number of samples analysed in a season}} \times 100$$

## RESULTS AND DISCUSSION

An analysis of 885 fecal samples, encompassing both bolus and guano, indicated that *P. medius* engages in the consumption of plant materials from six genera across five distinct families. There were seasonal variations in dietary preferences, specifically; *Morus alba* from the Moraceae family was consumed at rates of 17.5 % during the summer and 3.13 % in the winter months. *Ficus racemosa* was consistently present throughout the year, with its consumption peaking during the monsoon season at 54.35%. Additionally, *Artocarpus lacucha* (4.61 %) was observed during the monsoon, while *Ficus benjamina*

(37.33%) was predominantly consumed in the post-monsoon period. *Neolamarckia cadamba*, belonging to the Rubiaceae family, was identified in both summer (13.33%) and winter (28.75%), whereas *Mangifera indica* (30%) from the Anacardiaceae family was also consumed in the summer. *Psidium guajava*, representing the Myrtaceae family (6.67 % in the monsoon and 4 % in the post-monsoon) and the *Tectona grandis* from the Lamiaceae family was observed in the post-monsoon (24%) season. Unidentified plant material was detected across all seasons, with a peak occurrence of 34.36 % in the monsoon period (Table 2).

The analysis of guano further illustrated the dietary flexibility of *P. medius*. The *Ficus religiosa* was consumed during the summer (13.33%), and winter (4.62%). *M. alba* exhibited peak consumption in the summer (30.67%) and winter (6.15%), while *F. racemosa* was present throughout the year, with the highest consumption in the monsoon (33%). *N. cadamba* was observed in winter (13.85%), and *P. guajava* was particularly dominant during the monsoon season (42%). Unidentified plant material was found in all the seasons with the highest proportion (49.23%) occurring in the winter (Table 3). *P. medius* exhibits significant dietary adaptability with *F. racemosa* serving a crucial role in its diet throughout the year.

**Statistics:** Using a Generalized Linear Model (GLM) with a gamma distribution and log link function, the bolus analysis showed a strong model fit, with a deviance/df ratio of 0.395 and an Akaike Information Criterion (AIC) of 145.927. The Wald Chi-Square test for plant species was highly significant ( $\chi^2 = 93.323$ ), indicating that species like *F. benjamina* (B = 2.228), *M. indica* (B = 2.316), and *F. racemosa* (B = 2.140) were frequently consumed, with *F. benjamina* having the highest mean frequency (35.94). Although the effect of

**Table 2.** Seasonal frequency of occurrence of plant species in the bolus of *P. medius*

Family	Plant name	Common name	Summer	Monsoon	Post-monsoon	Winter
Moraceae	<i>Ficus religiosa</i>	Sacred Fig	+ (14.17)	-	-	+ (7.5)
	<i>Morus alba</i>	Mulberry	+(17.5)	-	-	+ (3.13)
	<i>Ficus racemosa</i>	Cluster Fig	+(16.67)	+ (54.35)	+ (18.67)	+(48.12)
	<i>Ficus bengalensis</i>	Banyan Tree	+(4.17)	-	-	-
	<i>Artocarpus lacucha</i>	Monkey Jack	-	+ (4.61)	-	-
	<i>Ficus benjamina</i>	Weeping Fig	-	-	+ (37.33)	-
Rubiaceae	<i>Neolamarckia cadamba</i>	Kadamba Tree	+(13.33)	-	-	+(28.75)
Anacardiaceae	<i>Mangifera indica</i>	Mango Tree	+(30.00)	-	-	-
Myrtaceae	<i>Psidium guajava</i>	Guava Tree	-	+ (6.67)	+ (4.00)	-
Lamiaceae	<i>Tectona grandis</i>	Teak Tree	-	-	+ (24.00)	-
	Unidentified	-	+ (4.16)	+ (34.36)	+ (16.00)	+ (12.5)

Summer = March to May; Monsoon = June to September; Post-monsoon = October and November; Winter = December to February. Presence [+], Absence [-]. Frequency of occurrence of plant species given in parenthesis

**Table 3.** Seasonal frequency of occurrence of plant species in the guano of *P. medius*

Family	Plant name	Common name	Summer	Monsoon	Post-monsoon	Winter
Moraceae	<i>Ficus religiosa</i>	Sacred Fig	+ (13.33)	-	-	+ (4.62)
	<i>Morus alba</i>	Mulberry	+ (30.67)	-	-	+ (6.15)
	<i>Ficus racemosa</i>	Cluster Fig	+ (21.33)	+ (33.00)	+ (27.70)	+ (26.15)
	<i>Ficus bengalensis</i>	Banyan Tree	+ (10.67)	-	-	-
	<i>Ficus benjamina</i>	Weeping Fig	-	-	+ (26.15)	-
Rubiaceae	<i>Neolamarckia cadamba</i>	Kadamba Tree	+ (17.33)	-	-	+ (13.85)
Myrtaceae	<i>Psidium guajava</i>	Guava Tree	-	+ (42.00)	-	-
	Unidentified	-	+ (6.67)	+ (25.00)	+ (46.15)	+ (49.23)

See table 2 for details

seasons on the bolus frequency was not statistically significant, descriptive statistics suggested trends highest mean frequency with the monsoon season (20.90). Seasonal trends in diet were less pronounced in the bolus, some seasonal variation in plant availability may still influence the composition of the bolus. The diet of *P. medius* is significantly influenced by plant species, with species like *P. guajava*, *F. racemosa*, *F. benjamina* and *M. indica* playing key roles.

In contrast, the guano analysis, the model fit was adequate, with a Pearson chi-square/df ratio of 0.155 and a Pearson chi-square/df ratio of 0.142. The Wald Chi-Square test revealed that both plant species ( $\chi^2 = 191.647$ ) and seasons ( $\chi^2 = 17.159$ ) had significant effects on the frequency of plant species in the guano of *P. medius*. Specifically, *P. guajava* (B = 2.282) and *F. racemosa* (B = 2.136) were in significantly higher frequencies, with *P. guajava* showing the highest mean frequency (32.33). Seasonal effects were also highly significant, with monsoon (B = 0.603) and summer (B = 0.582) showing higher frequencies compared to winter, and monsoon exhibiting the highest mean frequency (17.34). This suggests a clear seasonal shift in plant consumption, with certain species like *P. guajava* and *F. racemosa* being more prevalent during monsoon and summer. Parry-Jones and Augee (2001) also reported that fruits in the droppings of bats occurred in low quantity in winter season as compare to summer season.

The comparison of bolus and guano analyses underscores dietary flexibility of *P. medius*. The consistent presence of *F. racemosa* and other moraceae members highlights their ecological significance for bat populations and forest regeneration, as noted by Basharat et al. (2021). Schmelitschek et al. (2009) mentioned the major dietary part of these bats are *Ficus spp.* belonging to the family moraceae. The dominance of the moraceae family, particularly *Ficus spp.* is consistent with Gulraiz et al. (2016) and Javid et al. (2016) and also known to form a core component of diet of fruit bat due to their nutritional richness and year-round availability (Wendeln et al., 2000, Epstein et

al., 2009, Aung and Htay 2019). The consumption of *T. grandis* may indicate a response to specific nutritional needs during post- monsoon; although it is uncommon in the diet of *P. medius* and highlights opportunistic feeding behaviour. The inclusion of multiple plant families, such as Rubiaceae, Myrtaceae, Anacardiaceae and Lamiaceae suggests a strategy to mitigate competition and optimize nutrient intake (Sudhakaran and Doss 2012). This nutritional flexibility is essential for their survival, particularly in regions where the availability of fruit fluctuates throughout the year (Ashwin and Jayakumar 2019; Tiwari et al., 2019).

## CONCLUSIONS

The study presents a comprehensive analysis of the evolving dietary preferences of *P. medius* in Lucknow, India. It underscores the significance of specific plant families, particularly Moraceae, in their diet. *F. racemosa* emerges as a crucial and reliable food source for *P. medius*, evidenced by its consistent consumption throughout the year. The inclusion of *P. guajava*, *N. cadamba*, and *M. alba* in their diet illustrates the species' adaptability to variations in resource availability. The dietary habits of *P. medius* are significantly influenced by seasonal changes in food availability. The study highlights the importance of conserving a diverse array of plant species to support fruit bats, thereby enhancing our understanding of the ecological role of *P. medius*. Future research should aim to extend the geographical scope of dietary analyses to comprise various regions within India and examine the influence of vegetation composition and local environmental factors on dietary preferences. Long-term monitoring of feeding behaviour, particularly in relation to habitat changes and climate change, is essential for understanding dietary impacts on *P. medius* populations. Moreover, genetic analysis of bolus and guano samples could provide more detailed insights into the food ecology of *P. medius*, including identifying less conspicuous or degraded plant species in their diet.

## ACKNOWLEDGEMENTS

Authors would like to express gratitude to the director of National Botanical Research Institute (CSIR-NBRI), Lucknow for granting permission to collect samples at their Banthara research station.

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## Diversity of Trees in Narikkode Hill, Kannur, Kerala

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**Abstract:** The present study identified a total of 88 taxa belonging to 73 genera and 37 families from Narikkode Hill, Kannur, Kerala. Among these, dicotyledons were represented by 35 families while monocotyledons and gymnosperms by one family each. Five species were endemic. The dominant families were Mimosaceae, Moraceae and Rutaceae (6 species each) followed by Anacardiaceae, Euphorbiaceae and Fabaceae (5 species each) and Meliaceae (4 species). This study helped in generating primary data on the diversity and distribution of trees in Narikkode Hill.

**Keywords:** Tree diversity, Endemic, Western Ghats, Narikkode Hill

Plants are essential for the planet and all living things including humans. They provide food, fiber, shelter, medicine, and fuel. The basic food for all organisms is produced by green plants, forming the critical base of the food chain in all ecosystems. Human beings have used plants as food, medicine, and for meeting other needs since ancient times (Fernando 2012, Numpulsuksant et al., 2021). Plants are considered as rich source of bioactive chemicals, and they may serve as an alternative source for various medicines. Secondary metabolites or phytochemicals from plants exhibit excellent pharmacological activities such as antioxidative, antiallergic, antibiotic, and anti-carcinogenic properties. They have supported human societies even before the establishment of agriculture by providing food, clothing, shelter, remedies, and poisons.

Trees are perennial woody plants typically characterized by a single main stem or trunk, supporting branches and leaves. They play a crucial role in the ecosystem, providing a wide range of environmental, social and economic benefits. Kumar et al. (2020) documented 221 tree species from Amarkutir of West Bengal. The 106 species of trees from tropical mixed dry deciduous forest landscape in the Nilgiri Biosphere Reserve of the Western Ghats were recorded (Anitha et al., 2010). Volga et al. (2013) reported 136 endemic trees of Western Ghats. Manoj et al. (2012) reveals 63 riparian tree species of Alakyam stream. Mastan et al. (2020) reported 97 tree species in Nithyapoojakona dry deciduous forest of Sri Lankamalleswara wildlife sanctuary, Southern Eastern Ghats. Devi et al. (2018) observed 125 tree species belonging to 90 genera and 46 families in tropical moist forests of Mizoram. Nayak and Sahoo (2020) reported 94 tree species belonging to 29 families in forests of Odisha and the most dominant

family was Fabaceae. Similarly, Roy (2020) recorded 88 species of Magnoliophyta from an urban green space in Purulia, West Bengal. A total of 992 trees from 66 species belonging to 31 families were recorded from Thodupuzha urban region of Kerala (Padmakumar et al., 2021).

The complete documentation of tree diversity is essential for formulating conservation strategies. Documentation of tree diversity in major ecosystems is either completed or ongoing. However, some areas remain unexplored. In this context, the present study has been undertaken to study and document the tree diversity of Narikkode Hill in Kannur district of Kerala.

### MATERIAL AND METHODS

**Study area:** Narikkode Hill is situated in Triprangottur Grama Panchayath and is located between 11.8044 N, 75.6841 E in the Kannur district of Kerala. It covers a total area of 32.39 sq.km. The terrain is hilly and possesses remnants of erstwhile forests. The total population of the area is 333, of which 254 people belong to the tribal category Kurichiya, and 79 people belong to the Christian community.

**Survey procedure:** Extensive field surveys were undertaken during 2021-2022 to document the diversity of tree species. Information pertaining to the diversity was collected and representative specimens were gathered. Data on the uses of trees, along with its edible parts, medicinal parts, and the ailments they treat, were collected through surveys from tribal people residing in the study area. Plants collected during the present study were identified using floras (Hooker 1872-1897, Gamble 1915-1936, Ramachandran & Nair 1988, Sasidharan 2004), journals, and other relevant literature. Voucher specimens were prepared following routine herbarium

methods (Fosberg and Sachet 1965, Jain and Rao 1976) and deposited in Mahatma Gandhi Government Arts and Science College Herbarium. Photographs of live specimens were also taken from the field. The identity of doubtful specimens was confirmed at the Central National Herbarium (CAL), Calicut University (CALI), and Botanical Survey of India, Southern Regional Centre, Coimbatore (MH).

## RESULTS AND DISCUSSION

The study area is hilly and encompasses a variety of vegetation types such as semievergreen forests, dry deciduous forests and grasslands. The area exhibits degraded habitats due to anthropogenic activities such as agriculture, grazing etc. These diverse habitats contribute to the ecological richness of Narikkode hill in Kannur district.

The present study has recorded 88 taxa belonging to 73 genera and 37 families. The families were arranged according to Bentham and Hooker's system of classification (Bentham and Hooker, 1862-1883). Among these, 84

species were dicotyledons, 3 were monocotyledons, and one species to gymnosperm. The monocotyledons include *Cocos nucifera* L., *Areca catechu* L., and *Arenga wightii* Griff belonging to Arecaceae. The gymnosperm reported from the study area is *Araucaria heterophylla* (Salisb.) Franco belonging to Araucariaceae. The dominant families were Mimosaceae, Moraceae, Rutaceae, Anacardiaceae, Euphorbiaceae, Fabaceae and Meliaceae. The present study recorded 5 endemic species viz., *Arenga wightii* Griff., *Lagerstroemia microcarpa* Hance, *Vateria indica* L. which are endemic to Western Ghats and *Holigarna arnottiana* Hook.f., *Hopea parviflora* Bedd. which are endemic to Southern Western Ghats. Out of the 88 taxa recorded, 48 are utilized by the people to cure various ailments, and 24 are consumed in various ways on a day-to-day basis. A total of 57 tree species from the study area belongs to various IUCN categories (Table 1).

Among these, 24 taxa were observed in sacred groves, such as *Alstonia scholaris* (L.) R. Br., *Artocarpus*

**Table 1.** List of tree species

Binomial	Family	Local name	Phenology	Uses	Edible part	Medicinal parts	Ailments treated	IUCN Status
<i>Acacia catechu</i> (L.) Willd.	Mimosaceae	Karingali	March-September	Medicinal	-	Bark	Cough, Ulcer	LC
<i>Aegle marmelos</i> (L.) Correa.	Rutaceae	Koovalam	March-May	Medicinal, Edible	Fruit	Leaves	Fever, Nausea, Vomiting, Swellings, Dysentery	NT
<i>Albizia chinensis</i> (Osbeck) Merr.	Mimosaceae	Vaaka	March-July	-	-	-	-	-
<i>A. lebeck</i> (L.) Benth.	Mimosaceae	Peelivaka	March-December	Medicinal	-	Bark	Asthma, Cold, Cough	LC
<i>A. odoratissima</i> (L. f.) Benth.	Mimosaceae	Irumbukunni	April-January	-	-	-	-	LC
<i>Alstonia scholaris</i> (L.) R. Br.	Apocynaceae	Ezhilampala	October-February	Medicinal	-	Bark	Skin diseases, Rheumatism	LC
<i>Anacardium occidentale</i> L.	Anacardiaceae	Kashumavu	November-April	Medicinal, Edible	Fruit	Bark	Skin diseases	LC
<i>Annona reticulata</i> L.	Annonaceae	Aathachakka	May-August	Medicinal, Edible	Fruit	Seed	Diarrhoea	LC
<i>Araucaria heterophylla</i> (Salisb.) Franco.	Araucariaceae	Island pine	Throughout the year	-	-	-	-	VU
<i>Areca catechu</i> L.	Arecaceae	Kamuku	Throughout the year	Medicinal	-	Seed	Bronchitis, Obesity, Anaemia, Leprosy	-
<i>Arenga wightii</i> Griff.	Arecaceae	Malanthengu	July-September	-	-	-	-	VU
<i>Artocarpus incisus</i> (Thunb.) L.f.	Moraceae	Kadaplavu	January-June	Edible	Fruit	-	-	-
<i>A. heterophyllus</i> Lam.	Moraceae	Plavu	November-April	Edible	Fruit	-	-	-
<i>Averrhoa bilimbi</i> L.	Averrhoaceae	Bilumbi	March-May	Edible	Fruit	-	-	-
<i>A. carambola</i> L.	Averrhoaceae	Chathurappuli	May-August	Edible	Fruit	-	-	-
<i>Azadirachta indica</i> A. Juss.	Meliaceae	Veppu	February-September	Medicinal	-	Leaves, Flower, Seed	Fever, Skin diseases	LC

Cont...

**Table 1.** List of tree species

Binomial	Family	Local name	Phenology	Uses	Edible part	Medicinal parts	Ailments treated	IUCN Status
<i>Bischofia javanica</i> Blume	Euphorbiaceae	Cholavenga	March-October	-	-	-	-	LC
<i>Bridelia retusa</i> (L.) A. Juss.	Euphorbiaceae	Mulluvenga	August-December	-	-	-	-	LC
<i>Caesalpinia sappan</i> L.	Caesalpinaceae	Sappannam	August-December	Medicinal	-	Whole plant	Diarrhoea, Dysentery	LC
<i>Calophyllum inophyllum</i> L.	Clusiaceae	Punna	December-January	Medicinal	-	Leaves, Flower	Ulcer, Rheumatism	LC
<i>Careya arborea</i> Roxb.	Lecythidaceae	Aalam	February-July	Medicinal	-	Bark	Bronchitis, Skin disease	-
<i>Carica papaya</i> L.	Caricaceae	Pappaya	Throughout the year	Medicinal, Edible	Fruit	Bark, Leaves	Arthritis	DD
<i>Cassia fistula</i> L.	Caesalpinaceae	Konna	February-September	Medicinal	-	Root, Leaves	Relieving pain, Edema, Skin irritation	LC
<i>Casuarina littorea</i> Oken	Casuarinaceae	Kattadi	July-September	-	-	-	-	-
<i>Chrysophyllum roxburghii</i> G. Don	Sapotaceae	Pulichakka	April-November	-	-	-	-	LC
<i>Cinnamomum verum</i> J. Presl	Lauraceae	Edana	March-April	Medicinal	-	Whole plant	Asthma, Diarrhoea	-
<i>Citrus aurantium</i> L.	Rutaceae	Maduranaragam	October-December	-	-	-	-	-
<i>Citrus maxima</i> (Burm.) Merr.	Rutaceae	Babloos	April-November	Edible	Fruit	-	-	LC
<i>C. medica</i> L.	Rutaceae	Madulungam	November-April	Medicinal	-	Fruit	Sore throat, Cough, Asthma	LC
<i>Cocos nucifera</i> L.	Arecaceae	Thengu	Throughout the year	Medicinal, Edible	Fruit	Fruit	Arthritis, Diarrhoea	-
<i>Coffea arabica</i> L.	Rubiaceae	Kaappi	March-December	-	-	-	-	-
<i>Dalbergia latifolia</i> Roxb.	Fabaceae	Eeti	August-September	-	-	-	-	VU
<i>Erythrina variegata</i> L.	Fabaceae	Murikku	March-April	Medicinal	-	Bark, Leaves	Joint pain	LC
<i>Ficus exasperata</i> Vahl.	Moraceae	Parakam	February-April	-	-	-	-	LC
<i>F. hispida</i> L.f.	Moraceae	Kattathi	September-May	-	-	-	-	LC
<i>F. microcarpa</i> L.f.	Moraceae	Ithi	March-May	Medicinal	-	Leaves	Liver disease	LC
<i>F. racemosa</i> L.	Moraceae	Athi	February-May	Medicinal	-	Leaves	Diabetes, Liver disorder, Diarrhoea	LC
<i>Flacourtia jangomas</i> (Lour.) Raeusch.	Flacourtiaceae	Lavalolikka	November-April	Medicinal, Edible	Fruit	Root, Leaves	Bronchitis, Toothache	-
<i>Garcinia mangostana</i> L.	Clusiaceae	Mangosteen	Throughout the year	Edible	Fruit	-	-	-
<i>Gliricidia sepium</i> (Jacq.) Kunth	Fabaceae	Seemakonna	March-May	-	-	-	-	LC
<i>Gmelina arborea</i> Roxb. ex Sm.	Verbenaceae	Kumizhu	January-June	Medicinal	-	Whole plant	Fever, Thirst, Piles	LC
<i>Grevillea robusta</i> A. Cunn. ex R. Br.	Proteaceae	Silveroak	January-June	-	-	-	-	-
<i>Grewia tiliifolia</i> Vahl	Tiliaceae	Chadachi	June-September	Medicinal, Edible	Fruit	Bark, Root	Diarrhoea, Skin diseases	LC
<i>Hevea brasiliensis</i> (Willd. ex Juss.) Müll.-Arg.	Euphorbiaceae	Rubber	February-June	-	-	-	-	LC

Cont...

**Table 1.** List of tree species

Binomial	Family	Local name	Phenology	Uses	Edible part	Medicinal parts	Ailments treated	IUCN Status
<i>Holigarna amottiana</i> Hook.f.	Anacardiaceae	Cheru	January-July	Medicinal	-	Leaves	Inflammation, Arthritis, Obesity	-
<i>Hopea parviflora</i> Bedd.	Dipterocarpaceae	Thambakam	January-June	-	-	-	-	LC
<i>Lagerstroemia microcarpa</i> Hance	Lythraceae	Venthekku	June-February	-	-	-	-	-
<i>Lannea coromandelica</i> (Houtt.) Merr.	Anacardiaceae	Karayam	January-May	Medicinal	-	Leaves	Hepatitis, Diabetes	LC
<i>Macaranga peltata</i> (Roxb.) Müll.-Arg.	Euphorbiaceae	Uppila	October-December	-	-	-	-	LC
<i>Mallotus philippensis</i> (Lam.) Müll.-Arg.	Euphorbiaceae	Sinduri	October-March	Medicinal	-	Bark, Leaves	Skin problem, Bronchitis, Diabetes, Jaundice	LC
<i>Mangifera indica</i> L.	Anacardiaceae	Mavu	January-May	Edible	Fruit	-	-	DD
<i>Manilkara zapota</i> (L.) P. Royen	Sapotaceae	Sapota	February-June	Edible	Fruit	-	-	LC
<i>Michelia champaca</i> L.	Magnoliaceae	Chembakam	March-July	Medicinal	-	Root, Bark, Flower	Cough, Bronchitis, Hypertension, Dyspepsia,	LC
<i>Melia dubia</i> Cav.	Meliaceae	Valiyaveppu	March-February	-	-	-	-	-
<i>Miliusa tomentosa</i> (Roxb.) Finet & Gagnep.	nonaceae	Malaveppu	October-May	-	-	-	-	LC
<i>Moringa pterygosperma</i> Gaertn.	Moringaceae	Muringa	November-March	Medicinal, Edible	Fruit, Leaves	Bark, Leaves, Flower, Fruit	Arthritis, Rheumatism	-
<i>Murraya koenigii</i> (L.) Spreng.	Rutaceae	Kariveppila	March-July	Medicinal	-	Root, Leaves	Piles, Inflammation, Itching, Body aches	LC
<i>Myristica beddomei</i> King	Myristicaceae	Pathiri	December-May	-	-	-	-	-
<i>M. fragrans</i> Houtt.	Myristicaceae	Jaathikka	December-August	Medicinal	-	Fruit	Anxiety, Nausea, Cholera, Stomach cramps	DD
<i>Olea dioica</i> Roxb.	Oleaceae	Edala	November-April	Medicinal	-	Whole plant	Skin diseases, Rheumatism	-
<i>Persea macrantha</i> (Nees) Kosterm.	Lauraceae	Kulamavu	December-May	Medicinal	-	Leaf, Bark	Fracture, Asthma	-
<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Nelli	July-February	Medicinal, Edible	Fruit	Fruit	Jaundice, Inflammation.	LC
<i>Pongamia pinnata</i> (L.) Pierre	Fabaceae	Ungu	April-December	Medicinal	-	Whole plant	Piles, Skin diseases, Wound	LC
<i>Psidium guajava</i> L.	Myrtaceae	Perakka	March-May	Edible	Fruit	-	-	LC
<i>Pterocarpus marsupium</i> Roxb.	Fabaceae	Venga	September-October	Medicinal	-	Whole plant	Boils, Sores	NT
<i>Santalum album</i> L.	Santalaceae	Chandhanam	November-December	Medicinal	-	Bark	Bronchitis, Cystitis	VU
<i>Sapindus trifoliatus</i> L.	Sapindaceae	Soapinkaimaram	December-April	-	-	-	-	-
<i>Schleichera oleosa</i> (Lour.) Oken	Sapindaceae	Poovam	March-June	Medicinal	-	Bark	Skin inflammation, Ulcer, Itching, Acne	LC
<i>Spondias pinnata</i> (L.f.) Kurz	Anacardiaceae	Ambazham	March-December	Medicinal, Edible	Fruit	Bark	Joint pain, Diarrhoea, Dysentery, Vomiting	-
<i>Sterculia guttata</i> Roxb.	Sterculiaceae	Peenari	September-March	Medicinal	-	Leaf, Bark	Rheumatism	-

Cont...

**Table 1.** List of tree species

Binomial	Family	Local name	Phenology	Uses	Edible part	Medicinal parts	Ailments treated	IUCN Status
<i>Stereospermum colais</i> (Buch. -Ham. ex Dillwyn) Mabb.	Bignoniaceae	Poopathiri	February-October	Medicinal	-	Root	Piles, Nervous disorders	-
<i>Strychnos nux-vomica</i> L.	Loganiaceae	Kanjiram	March-December	Medicinal	-	Whole plant	Swelling of stomach, Constipation, Anxiety, Migraine	-
<i>Swietenia macrophylla</i> King	Meliaceae	Mahagony	April-March	-	-	-	-	EN
<i>S. mahagoni</i> (L.) Jacq.	Meliaceae	Mahagony	April-November	-	-	-	-	NT
<i>Syzygium aromaticum</i> (L.) Merr. & L.M. Perry	Myrtaceae	Karampu	December-April	Medicinal, Edible	Flower bud	Flower bud	Scabies, Cholera, Malaria, Tuberculosis	-
<i>S. cumini</i> (L.) Skeels	Myrtaceae	Njerakka	December-April	Medicinal, Edible	Fruit	Bark	Sore throat, Bronchitis, Asthma, Thirst	LC
<i>S. samarangense</i> (Blume) Merr. & L.M. Perry	Myrtaceae	Chambakka	February-June	Edible	Fruit	-	-	LC
<i>Tamarindus indica</i> L.	Caesalpiniaceae	Puli	September-April	Medicinal, Edible	Fruit	Fruit	Inflammation, Stomach pain, Throat pain, Rheumatism	LC
<i>Tectona grandis</i> L.f.	Verbenaceae	Thekku	May-January	Medicinal	-	Bark	Bronchitis, Constipation, Hyperacidity, Dysentery	EN
<i>Terminalia catappa</i> L.	Combretaceae	Bhadham	March-January	-	-	-	-	LC
<i>T. cuneata</i> B.Heyne ex Roth	Combretaceae	Neermaruthu	November-June	Medicinal	-	Bark	Fractures, Hemorrhage, Bronchitis, Diarrhoea	-
<i>T. paniculata</i> B.Heyne ex Roth	Combretaceae	Venmaruthu	August-February	-	-	-	-	-
<i>Theobroma cacao</i> L.	Sterculiaceae	Kokko	November-May	Edible	Fruit	-	-	-
<i>Vateria indica</i> L.	Dipterocarpaceae	Vellappayin	March-August	Medicinal	-	Bark	Chronic bronchitis, Throat troubles	VU
<i>Vitex altissima</i> L.f.	Verbenaceae	Mylellu	March-July	-	-	-	-	LC
<i>Wrightia tinctoria</i> B.Heyne ex Roth	Apocynaceae	Dhanthapala	February-November	Medicinal	-	Bark	PilSkin diseases	LC
<i>Xylia xylocarpa</i> (Roxb.) W. Theob.	Mimosaceae	Irumullu	February-December	Medicinal	-	Bark	Leprosy, Wound healing, Gonorrhoea, Rheumatism, Anaemia	LC
<i>Zanthoxylum rhetsa</i> (Roxb.) DC.	Rutaceae	Mullilam	March-November	-	-	-	-	LC

EN- Endangered; VU- Vulnerable; NT- Near Threatened; LC- Least Concern; DD- Data Deficient

*heterophyllum* Lam., *Careya arborea* Roxb., *Cassia fistula* L., *Chrysophyllum roxburghii* G. Don, *Cinnamomum verum* J. Presl, *Citrus medica* L., *Dalbergia latifolia* Roxb., *Ficus hispida* L. f., *Holigarna arnotiana* Hook.f., *Macaranga peltata* (Roxb.) Müll.-Arg., *Michelia champaca* L., *Moringa pterygosperma* Gaertn., *Olea dioica* Roxb., *Persea macrantha* (Nees) Kosterm., *Phyllanthus emblica* L.,

*Sapindus trifoliatus* L., *Schleichera oleosa* (Lour.) Oken., *Sterculia guttata* Roxb., *Strychnos nux-vomica* L., *Vateria indica* L., *Vitex altissima* L.f., *Xylia xylocarpa* (Roxb.) W. Theob. and *Zanthoxylum rhetsa* (Roxb.) DC.

There were 27 exotic tree species reported from the study area. They include species such as *Acacia catechu* (L.) Willd., *Anacardium occidentale* L., *Annona reticulata* L.,

*Averrhoa bilimbi* L., *Averrhoa carambola* L., *Araucaria heterophylla* (Salisb.) Franco., *Caesalpinia sappan* L., *Carica papaya* L., *Casuarina littorea* Oken, *Citrus aurantium* L., *Citrus maxima* (Burm.) Merr., *Cocos nucifera* L., *Coffea arabica* L., *Flacourtia jangomas* (Lour.) Raeusch., *Garcinia mangostana* L., *Gliricidia sepium* (Jacq.) Kunth., *Grevillea robusta* A. Cunn. ex R. Br., *Hevea brasiliensis* (Willd. ex A. Juss.) Muell.-Arg., *Manilkara zapota* (L.) P. Royen, *Myristica fragrans* Houtt., *Psidium guajava* L., *Swietenia macrophylla* King, *Swietenia mahagoni* (L.) Jacq., *Syzygium aromaticum* (L.) Merr. & L.M. Perry, *Tamarindus indica* L., *Terminalia catappa* L. and *Theobroma cacao* L.

A total of 28 cultivated species were also reported. They include *Anacardium occidentale* L., *Annona reticulata* L., *Araucaria heterophylla* (Salisb.) Franco., *Areca catechu* L., *Artocarpus incisus* (Thunb.) L.f., *Averrhoa bilimbi* L., *A. carambola* L., *Careya arborea* Roxb., *Carica papaya* L., *Cinnamomum verum* J. Presl, *Citrus aurantium* L., *C. maxima* (Burm.) Merr., *C. medica* L., *Cocos nucifera* L., *Coffea arabica* L., *Erythrina variegata* L., *Flacourtia jangomas* (Lour.) Raeusch., *Garcinia mangostana* L., *Gliricidia sepium* (Jacq.) Kunth, *Hevea brasiliensis* (Willd. ex A. Juss.) Müll.-Arg., *Manilkara zapota* (L.) P. Royen, *Psidium guajava* L., *Santalum album* L., *Swietenia macrophylla* King, *S. mahagoni* (L.) Jacq., *Syzygium aromaticum* (L.) Merr. & L.M. Perry, *Tamarindus indica* L. and *Theobroma cacao* L.

The collected species have shown significant variations in their phenology, with flowering and fruiting times either advancing or delaying compared to those recorded in previous literature. Therefore, observing phenological changes in response to climate change is crucial for predicting future impacts. Large-diameter trees make up approximately half of the mature forest biomass globally. Their dynamics and sensitivities to environmental changes can significantly influence global forest carbon cycling (Lutz et al., 2018).

### CONCLUSION

The present study recorded 88 tree species from Narikkode Hill of Kannur, Kerala exhibiting fairly good diversity of trees. However, the anthropogenic pressures are increasing at an alarming rate. In order to conserve the tree species which are characteristic to their unique ecosystem, regular monitoring is essential. In addition, public awareness programmes, adoption of effective and suitable conservation measures (extensive afforestation programme using indigenous species and *ex situ* multiplication of rare species) need to be implemented to save the diversity of trees.

### AUTHORS CONTRIBUTION

Vismaya C. conducted the field survey, collected the

plants and their uses. Pradeep Kumar G. and Girish Kumar E. identified the plants. Anusree M.E. drafted the manuscript. Sasikala K. reviewed the manuscript and corrected.

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# Distillery Wastewater Management using Natural Coagulant for Sustainable Environment

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**Abstract:** In India distilleries have become a major source of pollution as 88% of its raw materials are converted into waste and discharged into the water bodies, causing water pollution. The present study determine physical and chemical parameters in the treatment process of distillery wastewater using natural coagulants and assessing by comparing the performance with chemical coagulant alum. The plant-based method was best for distillery wastewater treatment. The crude extracts obtained from natural coagulant *Hibiscus rosa-sinensis* followed by *Moringa oleifera* have manifested improved coagulation performance compared to commercial alum. It exhibited a good reduction in turbidity, sulphate, biological oxygen demand, and chemical oxygen demand.

**Keywords:** *Hibiscus rosa-sinensis*, *Moringa oleifera*, Distillery effluent, Natural coagulant

The waste created by agro-processing industries is one of the major environmental issues faced by the world today. One among them is ethanol-producing distilleries which is considered a major source of water and soil pollution. Distilleries are among the 17 most polluting industries listed by the Central Pollution Control Board of India (CPCB 2003). Currently, in India, 397 distilleries produce approximately  $3.25 \times 10^{10}$  liters of ethanol and generate approximately  $40.90 \times 10^{15}$  liters of spent wash per annum (Kumar and Chandra 2018). For every one liter of alcohol production 15 liters of spent wash is generated (Beltran et al., 2001). Spent wash discharged into land or water bodies without proper treatment leads to pollution. In aquatic forms, it reduces the penetration power of sunlight thus reducing the photosynthetic activity and dissolved oxygen content. The discharge into land bodies without adequate treatment inhibits seed germination and leads to the depletion of vegetation by reducing soil alkalinity and manganese availability (Chowdhary et al., 2017 b). Due to high pollution status of spent wash, Ministry of Environment, Forest, and Climate Change, Govt. of India, enumerated ethanol industries at the top among the "Red category" industries (Tewari et al., 2007).

Coagulation and flocculation are the most common techniques adapted for treating various types of wastewater, including industrial, municipal, and stream water. During the coagulation process, synthetic materials like ferrous sulfate ( $\text{Fe}(\text{SO}_4)$ ), aluminum sulfate or alum ( $\text{Al}_2(\text{SO}_4)_3$ ), and poly aluminum chloride (PAC) ( $\text{Al}_2(\text{OH})_3\text{Cl}_3$ )<sub>10</sub> are added to form precipitates by neutralizing the charged particles. Synthetic materials are non-efficient, and make water treatment more

expensive which is not affordable by developing countries (Yuliasri 2016).

Physio-chemical treatment is used to overcome the problem, but it can't overcome several issues like decolorization, BOD, COD, the outlet of hazardous by-products, etc. This leads to the demand for more cost-effective and more environmentally friendly methods to be opted for detoxifying the hazardous waste. By sustainable development and waste minimization issues, phytoremediation is a new technology of using plants as a natural and eco-friendly process for the treatment of industrial effluent. Natural coagulants are biodegradable and do not introduce harmful chemicals into the environment, making them more sustainable and less pollution compared to chemical coagulants. The *Moringa oleifera* used to treat acidic wastewater, it showed a good reduction in turbidity, colour, and COD by 98, 90.76 and 65.8 % , respectively (Desta and Bote 2021). Wagh et al. (2022) report that *M. oleifera* when used to treat synthetic dairy wastewater reduce turbidity by 95 % and colour by 94 % . The main objective of the current work was to analyse the role of the natural coagulants on physico-chemical parameters of distillery effluent.

## MATERIAL AND METHODS

The effluent was collected from one of the distilleries in Karnataka and was stored in plastic cans at 4°C till further use to assess various physico-chemical characteristics. The temperature and pH of the effluent were recorded at the time of sample collection, by using thermometer and pocket digital pH meter, respectively. Sulphate, turbidity, biological oxygen

demand, and chemical oxygen demand were estimated in the laboratory by standard methods (APHA 2005).

**Preparation of material:** The leaves of *Hibiscus rosa sinensis*, *Moringa oleifera*, *Pisum sativum*, and *Azadiracta indica* were freshly collected, cleaned, and dried in the shade, fine powder was prepared and stored in an air-tight container for further study.

**Preparation of plant crude extract:** The various plant extracts concentrations were prepared using 100 ml of 1M NaCl solution and the suspension was stirred using a magnetic stirrer for 15 min and filtered through Whatman paper No.3 to extract the coagulation active compound. Aluminium sulphate (alum) which was used as a control was prepared in different concentrations by dissolving in water.

**Physio-chemical analysis of samples:** The distillery effluent collected from the industries, was subjected to study of physio-chemical characteristics such as pH, turbidity, sulphate, BOD, and COD in the laboratory. Environmental agencies and analytical protocols, such as those by APHA (American Public Health Association) recommend storing wastewater samples at 4°C to maintain integrity before analysis was followed. The samples were analyzed within 48 hrs after collection to ensure the accuracy and reliability of the results. The average value for every coagulant dosage was recorded based on three replications.

## RESULTS AND DISCUSSION

**Effectiveness of natural and chemical coagulants:** The use of natural coagulants was more effective in treating distillery effluent compared to the chemical coagulant alum. Physio-

**Table 1.** Characteristics of distillery effluent before treatment

Parameters	Value
pH	7.71
Turbidity	1700 NTU
Sulphate	200 mg/l
BOD	14,500 mg/l
COD	37,060 mg/l

**Table 2.** Turbidity and BOD of distillery effluent treated with chemical and natural coagulant extracts

Coagulant	Various concentrations of plant extracts					
	1 g		2 g		3 g	
	Turbidity	BOD	Turbidity	BOD	Turbidity	BOD
Alum	1006.67±11.55	13466.67±57.73	900±0	11966.67±57.73	743±11.55	11566.67±57.73
<i>Moringa oleifera</i>	1000±0	12966.66±57.73	900±0	11966.67±50	711.67±10.40	11533.33±57.73
<i>Hibiscus sp.</i>	1166.66±57.73	13500±0	896.67±5.77	11933.33±57.73	711.67±10.40	11533.33±57.73
<i>Azadiracta indica</i>	1366.67±28.87	13516.66 ±28.86	1250±50	12966±57.73	1200±50	11850±50
<i>Pisum sativum</i>	1700±0	14266.66±57.7	1693.33±5.77	13916.66±76.37	1600±0	13633.33±57.73

chemical parameters before treatment is given in Table 1.

**Turbidity reduction:** Natural coagulant extracts of *Moringa oleifera*, *Azadiracta indica* and *Hibiscus* resulted in the reduction in pH similar to that of alum. The initial turbidity of raw wastewater was 700 NTU. *Hibiscus sp.* and *Moringa oleifera* showed a similar turbidity reduction of 58% at an optimum dosage of 3g, outperforming alum, which achieved 56% reduction at the same dosage. *Azadiracta indica* reduced turbidity by 29%, while *Pisum sativum* was the least effective (Table 2). The reduction in turbidity increases with increased dosage up to optimal dosage and after that turbidity reduction decreases. When the coagulant concentration surpasses the optimum dosage no more colloids are free as they have been already neutralized into participates so excess coagulant will increase turbidity in water as no more opposite charged colloidal particles are available.

**Biological oxygen demand:** The high BOD levels in distillery effluent indicate limited oxygen availability for aquatic life. In distillery effluent, the amount of BOD was too high. *Hibiscus* and *Azadiracta indica* was best with 18% reduction in BOD reduction, which was similar to that of chemical coagulant alum. *Moringa oleifera* and *Pisum* reduced BOD by 7 and 4% respectively (Table 2).

**Sulphate;** The conventional alum showed just a 65% reduction in sulphate (Table 3). It curbs sulphate in itself hence the reduction of sulphate is less when compared to *Hibiscus* and *Moringa oleifera* which showed 84% of sulphate reduction from the effluent followed by *Azadiracta indica* with 40% of reduction and *Pisum sativum* being the least.

**Chemical oxygen demand (COD):** COD is usually higher than BOD because more organic compounds can be chemically oxidized than biological oxidation, hence the distillery effluent had 37,060 mg/l of COD. *Hibiscus* exhibited 21% of COD reduction, which was better than Alum, which showed a 18% reduction followed by *M.oleifera* and *A.indica* with a 14% reduction, and *P.sativum* being the least with a 6% reduction (Table 3).



**Table 3.** Reduction in sulphates and COD of distillery effluent treated with chemical and natural coagulant

Coagulant	Various concentrations of plant extracts					
	1 g		2 g		3 g	
	Sulphates	COD	Sulphates	COD	Sulphates	COD
Alum	79.67±0.57	31880±26.45	85±0.00	30360±10.00	86.67±2.89	29470±1.000
<i>Moringa oleifera</i>	46±1.73	31998.33±2.88	40.67±1.15	31967±15.39	33.33±2.89	30368.33±7.63
<i>Hibiscus sp.</i>	45±0	30450±50	40.67±1.15	29429.33±21.00	33.66±1.154	28470±26.45
<i>Azadiracta indica</i>	128.33±2.89	32033.33±57.73	119±1.15	31962±10.81	115±5	30433.33±28.26
<i>Pisum sativum</i>	200±0	35426.66±68.06	196.33±1.52	34790±10	191.67±2.88	33566.66±15.27

### CONCLUSIONS

The study highlighted the effectiveness of *Hibiscus rosa-sinensis* extract as a natural coagulant, achieving significant reductions in BOD, COD, sulphate, and pH levels, followed by *Moringa oleifera*. The highest removal observed at a dosage of 3 grams per 100 ml of wastewater. The findings suggest that using plant-based extracts is more cost-effective and efficient alternative to chemical treatments for distillery effluents, allowing for significant reductions in treatment expenses.

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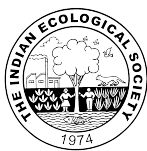
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Received 13 October, 2024; Accepted 24 January, 2025

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