ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)
Society reg : 131380
RNI No. : UPENG/2006/22736
NGO Darpan : UP/2016/0109266

Vol. 18, No. 1, January, 2023

Journal of Eco-Friendly Agriculture

Doctor's Krishi Evam Bagwani Vikas Sanstha
(Doctor's Agricultural and Horticultural Development Society)
Registered under Society Registration Act 21, 1860
Lucknow - 226 016, India

JOURNAL OF ECO-FRIENDLY AGRICULTURE

(A Bi-annual Scientific Research Journal)
Doctor's Krishi Evam Bagwani Vikas Santha
(Doctor's Agricultural and Horticultural Development Society)
Registration No. ISSN : 2229-628X (print), eISSN 2582-2683 (online)

ANNUAL MEMBERSHIP FEE EFFECTIVE FROM JANUARY, 2023

	Indian Individual (INR)	Indian Institution (INR)	Foreign Individual (US$)	Foreign Institution (US$)
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Processing Charges	2000.00 Per article	Not Applicable	50.00 per article	Not Applicable
Life Membership	10000.00	18000.00	1000.00	2500.00

Owner: *Doctor's Krishi Evam Bagwani Vikas Sanstha, Publisher: Dr. Ram Prakash Srivastava, 108, Lekhraj Khazana, Faizabad Road, Lucknow, Printed at: Army Printing Press: 33, Nehru Road, Sadar, Cantt. Lucknow.*

ISSN : 2229-628X (print)
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Vol. 18 **No. 1** **January, 2023**

Acknowledgment : DKEBVS acknowledges the financial support from ICAR and SERB, New Delhi
Journal is Accredited by NAAS - ICAR rating 5.23

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पत्रांक : 1270 / PSDG / 2022
Letter No. :

दिनांक : 15 / 12 / 2022
Date :

Message

Natural Farming is a chemical-free farming system rooted in Indian tradition enriched with modern understanding of ecology, resource recycling and on-farm resource optimization. It is considered as agroecology based diversified farming system which integrates crops, trees and livestock with functional biodiversity. It is largely based on on-farm biomass recycling with major stress on biomassmulching, use of on-farm cow dung-urine formulations; maintaining soil aeration and exclusion of all synthetic chemical inputs. Natural farming is expected to reduce dependency on purchased inputs. It is considered as a cost-effective farming practice with scope for increasing employment and rural development. Union Minister of Agriculture announced that to promote natural farming, the central government has approved the National Mission on Natural Farming as a separate scheme with an expenditure of Rs 1,584 crore.

I am happy to learn that Doctor's Krishi Evam Bagwani Vikas Sanstha (Doctor's Agricultural and Horticultural Development Society) is publishing a Journal of Eco-Friendly Agriculture since 2006 for promoting the innovative technologies of horticultural and agricultural crops encompassing different aspects of organic and natural farming.

I congratulate the entire team of the society for promoting awareness about organic and natural farming.

(Sanjay Singh)

Journal of Eco-friendly Agriculture **18**(1): 1-10. 2023
doi: 10.5958/2582-2683.2023.00001.1

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Response of vermicompost leachate on seed germination and crop growth

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ABSTRACT

The experiment conducted with lettuce, tomato and maize seeds pre-soaked in 5, 10, 20 per cent vermicompost concentrations and for 10 hrs and supplemented with NPK fertilizer showed that vermicompost at 10 per cent concentration produced maximum seed germination in lettuce and tomato, while maximum germination in maize seeds was obtained with 20 per cent vermicompost concentration. There was a significant difference in the number of leaves, length of shoot, length of root, wet and dry weight of plants tested at ($P<0.01$) across treatments. It was concluded that pre-soaking seeds in vermicompost leachate was beneficial in maximising plant growth.

Keywords: Vermicompost, seed germination, plant growth

Agro-based research is essential to boost the crops production. Agriculture in developing countries is adversely affected due to insufficient technology and scientific research on the growth particularly in monocotyledon and dicotyledonous plants (Esmaeilpour & Van Damme, 2016). The viability of seeds often increases the cost of production due to poor germination. Most of the seeds are erratic and undergo a high mortality rate under natural environmental conditions. Seed germination is a strategy for achieving efficient production (Maron *et al.*, 2013; Kattimani *et al.*, 1999). Pre-soaking reduces the lag phase in seed germination and using inorganic salt is a widely acceptable method to promote plant germination, growth, and development (Esmaeilpour & Van Damme, 2016). Seeds can be made viable at germination by pre-soaking or seed priming. Seed germination of several crop plants can be enriched by using chemical pre-treatments with KNO_3, KH_2PO_4, K_3PO_4, H_2O_2, salicylic acid in polyethylene glycol (SA, PEG) (Maron *et al.*, 2013; Kattimani *et al.*, 1999). Studies have proved that pre-soaking of seeds in inorganic solutions reduces the osmotic potential and contribute to salt accumulation in the seeds. It changes microbial diversity and the chemical properties of soil (Esmaeilpour & Van Damme, 2016) by promoting acidification and reducing nutrient uptake by the plant (Muhammad *et al.*, 2017). As a result, the development of the embryo is affected. In addition, cost it being lier is out of the reach of most farmers (Byrnes, 1990). However, pre-treatment of seeds with vermicompost, a non-chemical solution, has been implemented in many research studies. Soaking of seeds with vermicompost besides being eco-friendly improves the quality of seedlings, replacing the need of inorganic salts (Muhammad *et al.*, 2017). Use of inorganic salts are a traditionally acceptable and accessible method of providing a rich source of nutrients for plant growth. It is potentially viable and supports the activity of microbes that stimulate growth and development at the early seeding stage. Dicotyledonous plants germinate faster than monocotyledon plants under normal atmospheric conditions (Korkmaz & Pill, 2017). Lettuce and tomato belong to the former group but the yield and timing of harvest are affected by the emergent (Korkmaz & Pill, 2017). Maize, a monocotyledon plant takes some more time to germinate hence, attention should focus on pre-soaking in order to enhance germination. Emerging seedlings having direct contact with soil-borne pathogenic bacteria which prolong germination duration (Maron *et al.*, 2013). Pathogenesis caused by these kinds of harmful bacteria is less immutable against the development of seedlings. Other factors affecting plant development include moisture content in the soil due to evaporation (Fischer *et al.*, 2007), amount of light, oxygen, and other edaphic factors (Muhammad *et al.*, 2017). Different concentrations of vermicompost leachate/ tea have been used by researchers to determine the germination of various plants (Norman & Arancon, 2012). According to Norman & Arancon (2012), 1 per cent vermicompost tea increases seed germination whereas 5 per cent leachate significantly influences plant growth. It was also reported that low and moderate concentrations 1, 5, 10 and/or 20 per cent are appropriate for germination, depending on the species being grown (Gederts, 2011). There have been different schools of thought using different concentrations of vermicompost tea. However, to juxtapose the current knowledge on the appropriate

leachate concentration requirements for germination, the experiment on effect of pre-soaking of lettuce, tomato and maize seeds in vermicompost tea leachate compared to inorganic fertilizer was investigated.

MATERIALS AND METHODS

Certified homogenous seeds of lettuce (var Great lake), tomato (Roma VFN) and maize (Zama star) with batch number 60013630005095, 6001363005248 and 6001363005101, respectively were procured from Starke Ayres (Pty) Limited, Gauteng, South Africa. Vermicompost leachate tea extract, prepared from *Eisenia andreii* worms + cardboard + cow dung + grass cutting (www.wizzardworm.co.za) as organic fertiliser and NPK (3:1:6) 46 + trace element procured from Grovida Pty. Ltd. as inorganic fertilizer was used.

Seeds were treated separately with vermicompost leachate tea (VC) and inorganic (NPK) solution. About 200 seeds were pre-soaked in 100 ml of the following concentrations (0% deionized water, 5 per cent VC + 95 per cent deionized water, 10 per cent VC + 90 per cent deionized water, 20 per cent VC + 80 per cent deionized water) and an inorganic concentration (1 g salt + 1 *l* of deionized water) in 250 ml Erlenmeyer flasks for 10 hours. A control experiment with pre-soaked seeds in deionized water was also made in the same manner. The vermicompost leachate, inorganic solution, and water were then decanted. Seeds were allowed to dry thoroughly in whatman filter paper. Twenty-five seeds from each soaking treatment were kept in sterilized petri plates lined with whatman filter paper submerged with 5 ml of each treatment.

During germination setup, the seeds were placed with the endosperm within the moist filtered paper. The petri plates with each treatment were incubated in a cultured room at a temperature of 25±2 °C. The experiments were arranged in a completely randomized design for an equal chance of sprouting and germination. Five treatments based on concentrations with five replications per treatment were setup. Each treatment contained 25 seeds. Three to five seedlings were sampled from each replication. Seed emergence was recorded after two days as the emergence of the radicle. Seedlings were allowed to develop for 15 days. Seedlings from each petri plates were randomly selected for measuring shoot length, the number of leaves, root length, and the biomass production. Readings were performed using a calibrated ruler and flexible tape measure, where necessary.

Plant biomass measurements were accomplished by uprooting individual plants from each pot without causing any damage to the seedlings. Tap water was used to wash the seedlings thoroughly to remove soil and organic particles. A clear plastic ruler was used to measure the length of the shoot and the root of the uprooted plant in centimetres. Radwag electronic balance (PC1200C2l) was used to determine the fresh weight (g) of the whole plant parts (shoot, leaves, and root). Plant parts were dried in the oven at 60 °C for 24 hours and thereafter weighed to calculate the dry mass (g).

The pH of the vermicompost used was analyzed using a pH metre. The primary nutrients in the vermicompost leachate were determined at the laboratory unit at Cedara following the method outlined by Kulkarni *et al.* (2014). Vermicompost filtrate was diluted with de-ionized water in a ratio 5:20. ICP-OES (inductive coupled plasma atomic emission spectroscopy) was used to analyze the diluted solution for elements present. The resultant component was calibrated under standard measurement. The raw data obtained from the ICP-OES were subjected to further calculations. The dry matter obtained earlier as well as the sampled weight were used to determine the weight of the elements.

Table 2.1 Chemical properties of the vermicompost

Parameters	Measurement (unit)	Amount
C	%	11.07
S	%	0.19
N	Ppm	59.96
Ca	Ppm	97.60
Mg	Ppm	251.20
K	Ppm	781.20
Na	(mg. kg-1)	103.6
Zn	(mg. kg-1)	0.08
Cu	(mg. kg-1)	0.08
Mn	(mg. kg-1)	0.04
Fe	(mg. kg-1)	0.12
P	%	8
Moisture	%	99.87
pH	-	8.70

Analysis of variance of plant growth parameters and seed germination rates were performed using (SPSS model, 2019 version) trend analysis by a simple linear model. Statistical significance was determined at a 95 per cent confidence level.

RESULTS AND DISCUSSIONS

Responses of vermicompost leachate on lettuce seeds

No significant differences in lettuce seed germination with 0, 5 and 20 per cent vermicompost treatments was observed. However, there was a significant difference with 10 per cent treatment. In the 10 per cent treatment,

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00001.1

germination was higher than other treatments (Table 2.2). The rate of germination varied amongst the 0, 5, 10, 20 per cent and the NPK treated seeds. No difference was observed in the germination index among various treatments (Figure 2.1). The vigour of the lettuce seedlings varied with the different treatments and as a result plant mortality was inevitable. These attributes were more evident using 20 per cent and inorganic treatments. There was no significant different in the number of lettuce leaves observed in the 0, 5, and 20 per cent treatments. However, a significant difference in number of leaves were observed using the 10 per cent treatment (Table 2.2 & Figure 2.5b). Amongst the treatments, the 10 and 20 per cent vermicompost treatments resulted in a 5 per cent higher leaf production (1.46 ± 0.50^{ab}) compared to the other concentrations. The application of 10 per cent ($1.51 \text{ cm} \pm 0.32^{a}$) vermicompost concentration highly influenced the root length of lettuce plants followed by 5 per cent ($1.42 \text{ cm} \pm 0.22^{ab}$) untreated control ($1.28 \text{ cm} \pm 0.42^{b}$) and inorganic ($1.21 \text{ cm} \pm 0.21^{b}$) (Table 3.3 & Figure 3.5c). The 20 per cent treatment did not yield the same result. Plants with 10 per cent (2 ± 0.34^{a}) vermicompost concentration responded better than with inorganic treatment (1.78 ± 0.43^{ab}). The highest number of leaves were found in the 10 per cent (2 ± 0.34^{a}) vermicompost concentration (Table 2.3 & Figure 2.5a). The fresh weight was found to be higher in the 10 per cent ($0.02 \text{ g} \pm 0.02^{a}$) followed by 5, 20 per cent, inorganic and untreated control treatments; number of leaves in these treatments were similar (Figure 2.5d).

Response of vermicompost leachate on tomato seed

Tomato seed sprouting was observed in the petri dishes with vermicomposting extract four days after sowing (Figure 2.4). There was no significant difference is seed germination

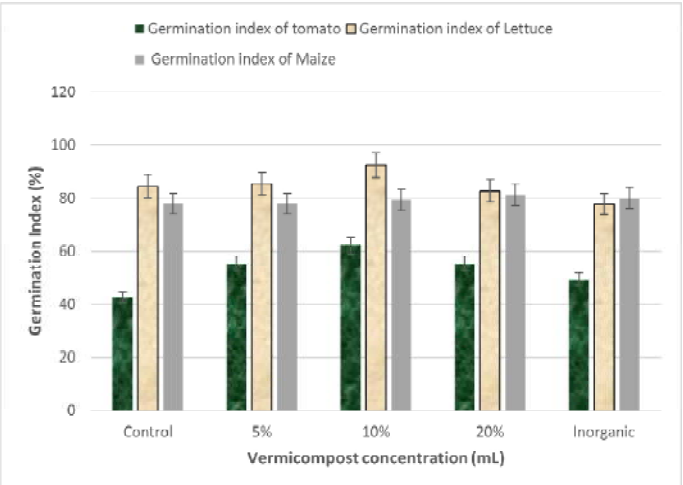

Figure 2.1: Lettuce, maize, and tomato seedling responses of vermicompost leachate N=5, mean ± SE

Table 2.2: Germination index of tomato, lettuce, and maize.

Treatment	Tomato	Lettuce	Maize
Control	42.67 ± 33.15	84.45 ± 22.18	78 ± 38.51
5%	55.33 ± 40.82	85.53 ± 6.19	78 ± 32.57
10%	62 ± 43.95	92.22 ± 19.03	79.33 ± 37.04
20%	55.33 ± 41.83	82.76 ± 8.79	81.33 ± 34.99
Inorganic	49.33 ± 38.33	77.78 ± 22.58	80 ± 33.37

N=5, mean ± SD (P>0.005)

Table 2.3: Response of vermicompost leachate on lettuce seedling

Treatment	Number of leaves	Plant height unit	Length of root unit	Wet weight of plant
Control	1.56 ± 0.51^{c}	1.22 ± 0.20^{b}	$1.28 \pm 0.0.42^{b}$	0.01 ± 0.002^{b}
5%	1.89 ± 0.32^{ab}	1.46 ± 0.50^{ab}	1.42 ± 0.22^{ab}	0.02 ± 0.02^{a}
10%	2 ± 0.343^{a}	1.72 ± 0.50^{a}	1.51 ± 0.32^{a}	0.01 ± 0.003^{b}
20%	1.61 ± 0.50^{bc}	1.68 ± 0.46^{a}	1.25 ± 0.28^{b}	0.01 ± 0.003^{b}
Inorganic (NPK)	1.78 ± 0.43^{ab}	1.26 ± 0.22^{b}	1.21 ± 0.21^{b}	0.01 ± 0.001^{b}

Table 2.4: Response of vermicompost leachate on tomato seedlings

Treatments	Number of leaves	Root length	Shoot length	Wet weight	Dry weight
Control	1.67 ± 0.48^{c}	3.63 ± 1.03^{bc}	4.44 ± 1.22^{c}	0.040 ± 0.018^{b}	0.036 ± 0.0
5%	1.9 ± 0.3^{ab}	4.51 ± 1.79^{ab}	6.24 ± 0.96^{a}	$0.048 \pm .016^{ab}$	0.06 ± 0.0
10%	2 ± 0.0^{a}	4.7 ± 0.98^{a}	6.27 ± 0.92^{a}	0.056 ± 0.012^{a}	0.045 ± 0.0
20%	1.71 ± 0.46^{b}	3.78 ± 1.84^{bc}	5.32 ± 1.53^{b}	$0.050 \pm .014^{ab}$	0.045 ± 0.0
Inorganic (NPK)	1.57 ± 0.51^{c}	3.19 ± 0.95^{c}	4 ± 0.95^{c}	0.042 ± 0.018^{b}	0.017 ± 0.0

using the 0, 5 and 20 per cent treatments but there was a significant difference using the 10 per cent treatment. The inorganic treatment (49.33% ± 38.33) was higher than the untreated control (42.67% ± 33.15) (Table 2.2). The vigour of seedlings were low in the 0, 5 and 20 per cent treatments but was significantly higher in the 10 per cent vermicompost treatment. The surface curve obtained from the growth indicated that a minimum concentration of vermicompost may be required for the germination of tomato seedlings (Figure 2.4). It was observed that there was no difference in number of leaf and shoot length in 0 and 20% treatments. However, amount of leaf in 10% was significantly higher for tomato (Figure 2.6a &c). The untreated control (1.67 ± 0.48^{c}) and inorganic treatments (1.57 ± 0.51^{c}) had similar number of leaves (Table 2.4 & 2.6a). Furthermore, the 10 per cent vermicompost concentration produced a significant difference with root elongation ($4.7 \text{ cm} \pm 0.98^{a}$) at ($P<0.05$). The root elongation was least affected when seedlings were treated with the inorganic treated. (Table 2.4 & Figure 2.6c).

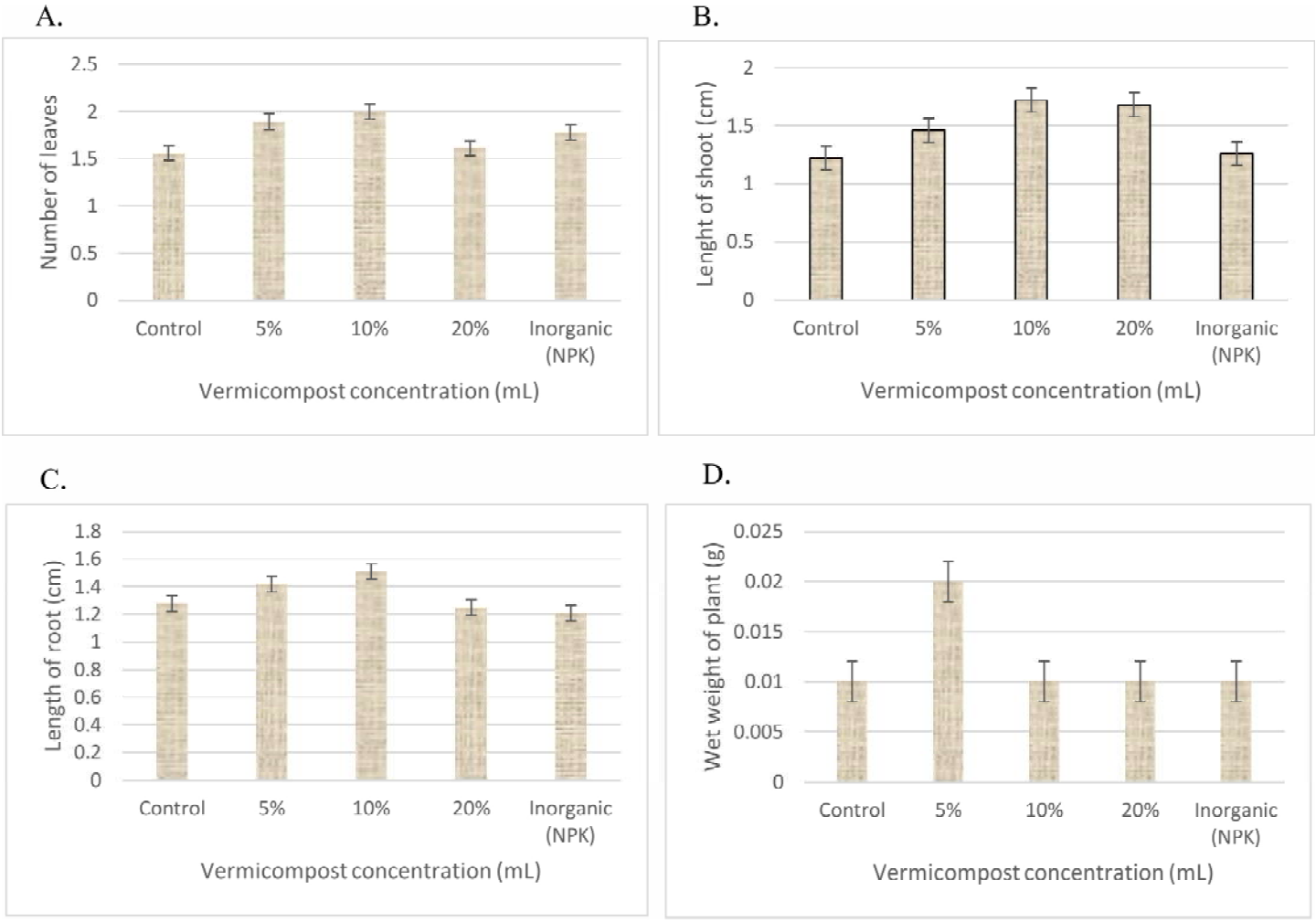

Figure 2.5: Lettuce germination responses of different vermicompost concentrations; A. Number of leaves B. Length of shoot (cm) C. Length of root (cm) D. N=5, Mean + S.E

The wet weight of the plant was significant at ($P<0.05$) (Table 2.4). Plants treated with the 10% vermicompost concentration presented the greatest wet weight ($0.056 \text{ g} \pm 0.012^{a}$) (Table 2.4 & Figure 2.6b). The same trend was observed with the untreated control ($0.040 \text{ g} \pm 0.018^{b}$) and the inorganic treated ($0.042 \text{ g} \pm 0.018^{b}$) treatments. The result obtained with inorganic fertilizer was not effective due to the accumulation of salt in the seed in the process of breaking the coating layer (Table 2.4, Figure 2.6).

Responses of vermicompost leachate on maize seed

Sprouting was observed in 5, 10, 20 per cent and the NPK treated solutions less than 48 hours after sowing, except in the untreated control (Figure 2.4 & Table 2.5). The highest and synchronized germination index (81.33% ± 34.99) was observed using the 20 per cent followed by the inorganic treated (80% ± 33.37) > 10 per cent vermicompost concentration (79.33% ± 37.04) > 5% (78% ± 32.57) (Table 2.2). The result obtained indicated that there was no significant difference in the rate of germination (Table 2.2). Furthermore, there were no significant differences in the number of leaves of maize in the 0, 5 and 10 per cent vermicompost concentrations. However, a significant difference in the number of leaves was recorded with 20 per cent VCL (Figure 2.7a). The shoot length was observed to be the highest with 20 per cent VCL (Table 2.5). Furthermore, 10 per cent VCL was found to significantly affect root length. Root length was not significant using the inorganic treatment (2.7c). There were no significant differences in the wet weight of leaves of maize in 0, 5, 10% and the inorganic treated concentrations. However, a significant difference was observed using the 20 per cent VCL (Figure 2.7d). Similarly,

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00001.1

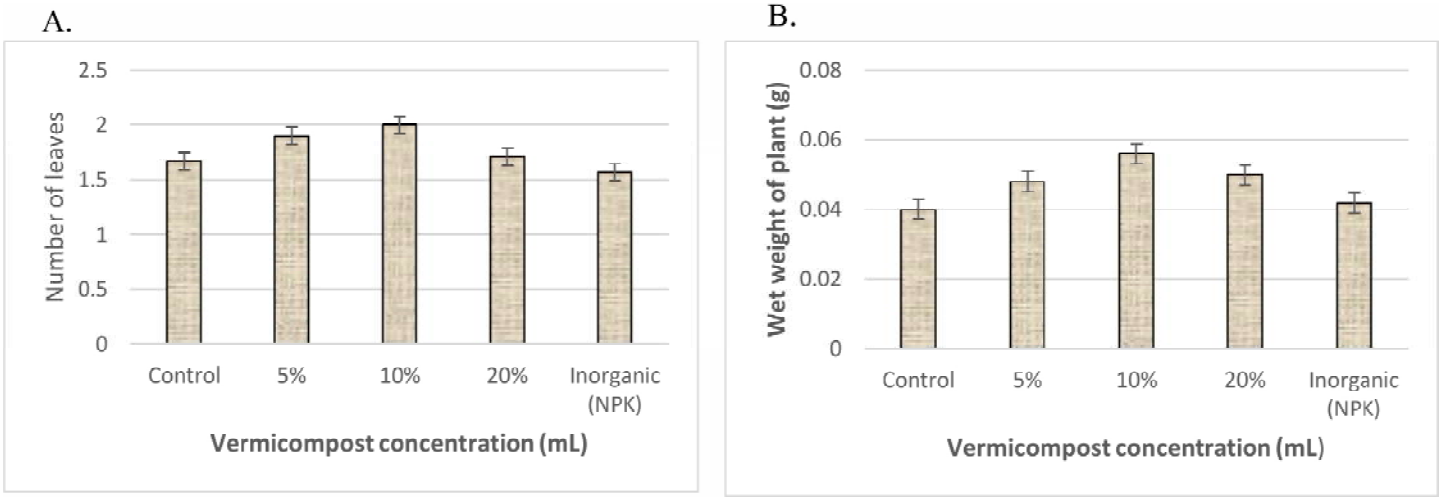

Figure 2.6: Tomato germination responses of different vermicompost concentrations; A. Number of leaves B. Wet weight of leaves (cm) C. Length of root (cm). D. Dry weight of plant E. Length of shoot. N = 5, mean ± S.E

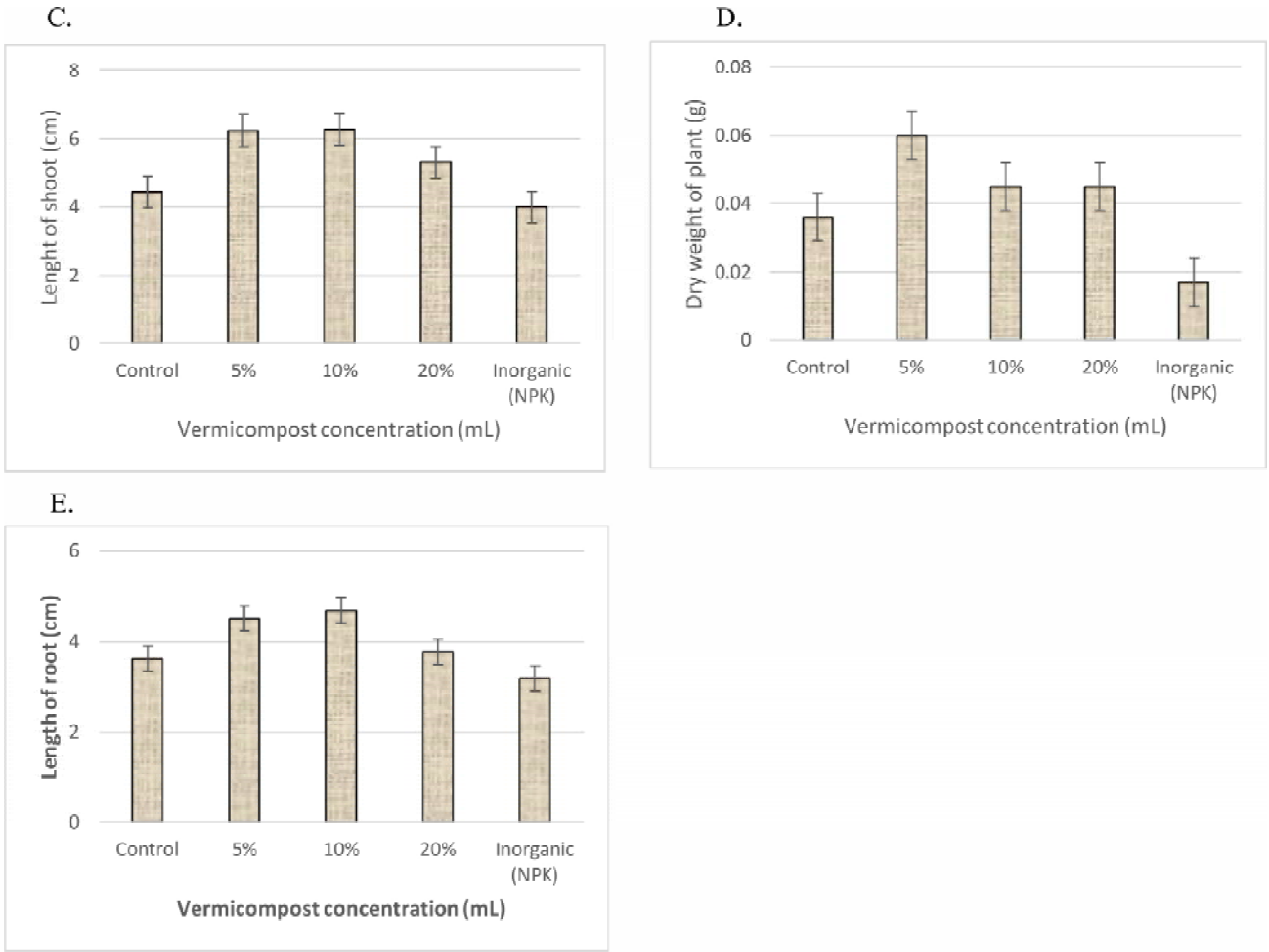

Figure 2.6: Tomato germination responses of different vermicompost concentrations; A. Number of leaves B. Wet weight of leaves (cm) C. Length of root (cm). D. Dry weight of plant E. Length of shoot. N = 5, mean ± S.E

Table 2.5: Responses of vermicompost leachate on maize seedlings

	Treatments				
Seedling parameters	**Control**	**5%**	**10%**	**20%**	**Inorganic (NPK)**
Number of leaves	1.58± 0.69^{b}	1.79 ± 0.71^{ab}	1.79 ± 0.54^{ab}	2.05 ± 0.4^{a}	1.63 ± 0.60^{b}
Plant height	3.62 ± 0.52^{c}	3.55 ± 0.76^{c}	4.14 ± 0.97^{b}	5.48 ± 0.71^{a}	3.74 ± 0.70^{bc}
Length of root	13.98 ± 1.53^{c}	17.68 ± 3.08^{ab}	18.87 ± 2.4^{a}	16.47 ± 2.86^{b}	10.68 ± 3.9^{d}
Wet weight of root	0.074 ±0.0.45^{c}	0.16 ±0.075^{b}	0.16 ± 0.75^{b}	0.23 ± 0.84^{a}	0.162 ± 0.64^{b}
Wet weight of shoot	0.086 ± 0.63^{c}	0.23 ± 0.65^{ab}	0.23± 0.060^{ab}	0.27 ± 0.8^{a}	0.21 ± 0.062^{b}
Wet weight of root	0.12 ± 0.051b^{c}	0.19 ± 0.097^{a}	0.19 ± 0.069^{a}	0.15 ± 0.067^{a}	0.081± 0.039^{c}
Dry weight of leave	0.0087± .004^{c}	0.015 ± 0.0031^{b}	0.015 ± 0.0058^{b}	0.023±0.0048^{a}	0.016± .0034^{b}
Dry weight of shoot	0.0054±0.0048^{c}	0.019 ± 0.062^{bc}	0.018 ± 0.061^{b}	0.024± .062^{ab}	0.02± .00510^{a}
Dry weight of root	0.012 ±0.003^{bc}	0.016 ±0.0054^{ab}	0.017 ± 0.0095^{a}	0.018 ± .0048^{a}	0.0079± .033^{c}

N=5, mean ± SD

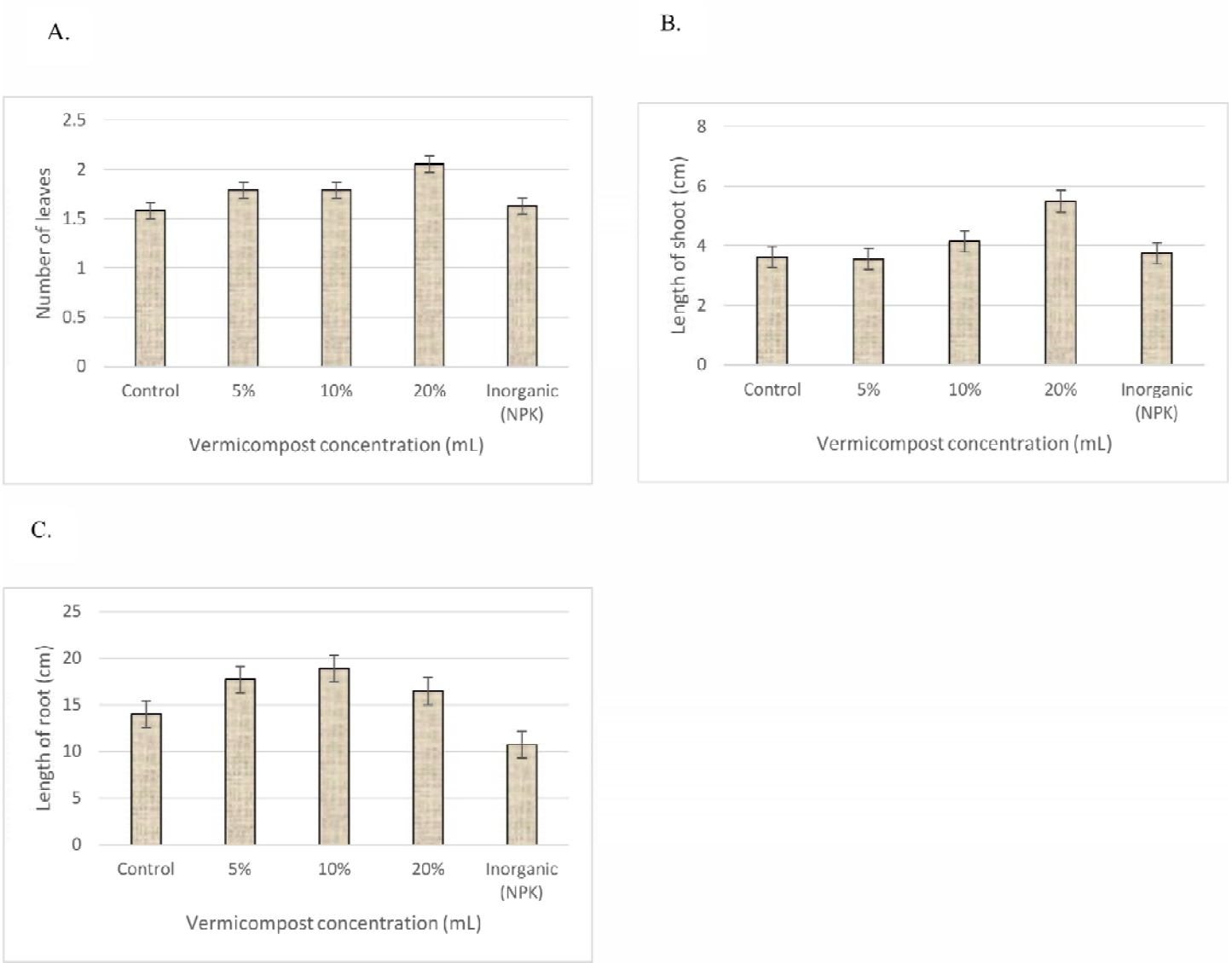

Figure 2.7: Maize germination responses of vermicompost leachate; A. Number of leaves B. Length of shoot (cm) C. Length of root (cm). N = 5, mean + S.E

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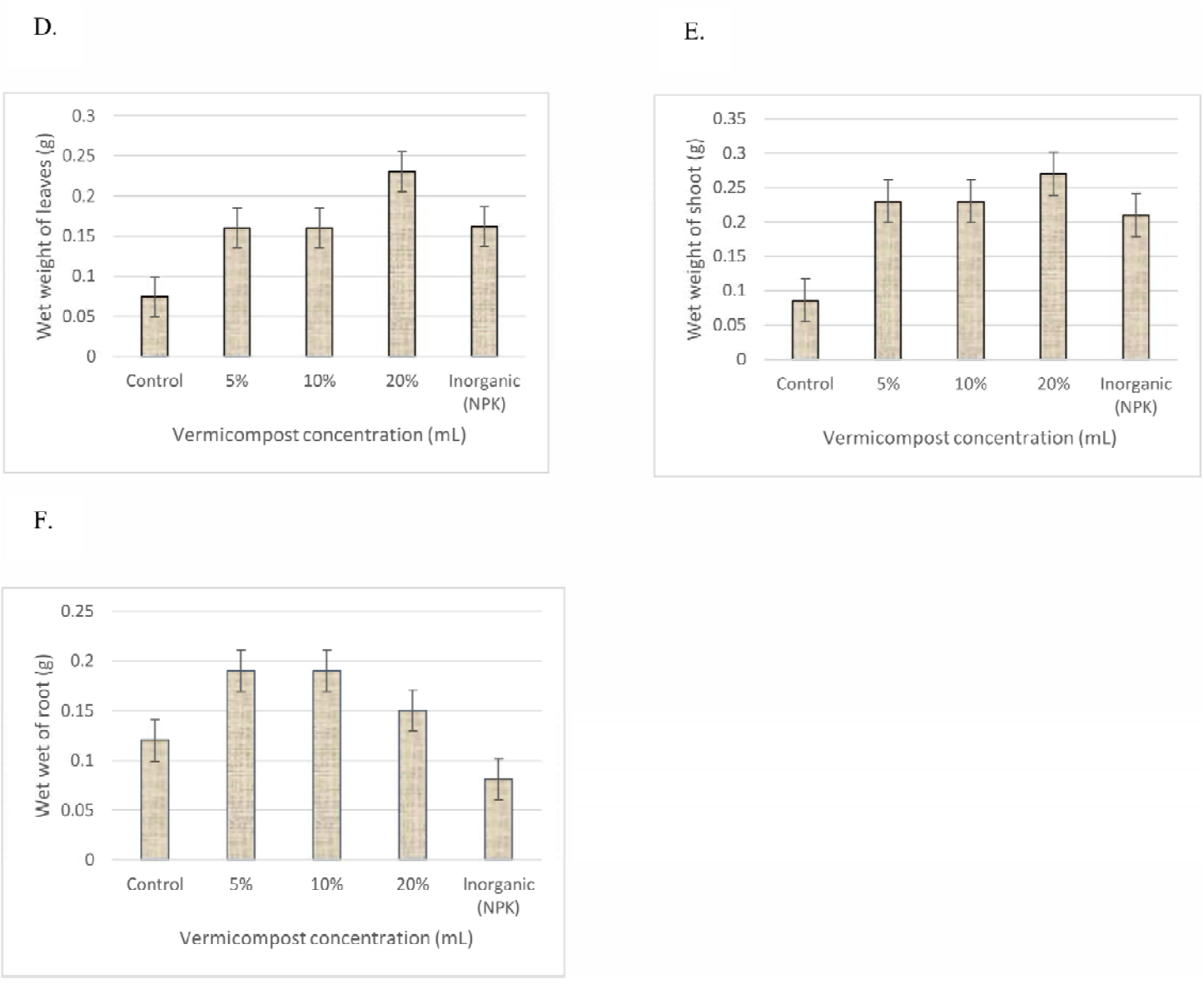

Figure 2.7: Maize germination responses of different vermicompost concentrations; D. Wet weight of leaves E. Wet weight of shoot F. Wet weight of root (cm) N=5, mean ± S.E

the wet weight of shoot exhibited the highest value at 20 per cent VCL (Figure 3.7e). The wet weight of root responded differently. The highest value with root length was observed using the 5 and 10 per cent VCL. Again, no significant differences were observed in root length using the inorganic treatment (Figure 2.7c). The dry weight of maize was significantly higher in the 20 per cent treatment compared to the rest. (Figure 2.7g). A similar trend was observed for the dry shoot weight (Figure 2.7h).

The germination and seedling emergence are the two critical phases for crop establishment. These phases are based on an internal regulator, environmental factors and/or hormonal stimulator and inhibitors (Kaur *et al.*, 2018). Pre-soaking is a metabolic activity that brings about softening of the hard seed coat. It plays a significant role in germination. In this case, sprouting is superimposed with seeds synchronized with pre-soaking. These speeds up the rate of germination. This is as a result of the release of germination

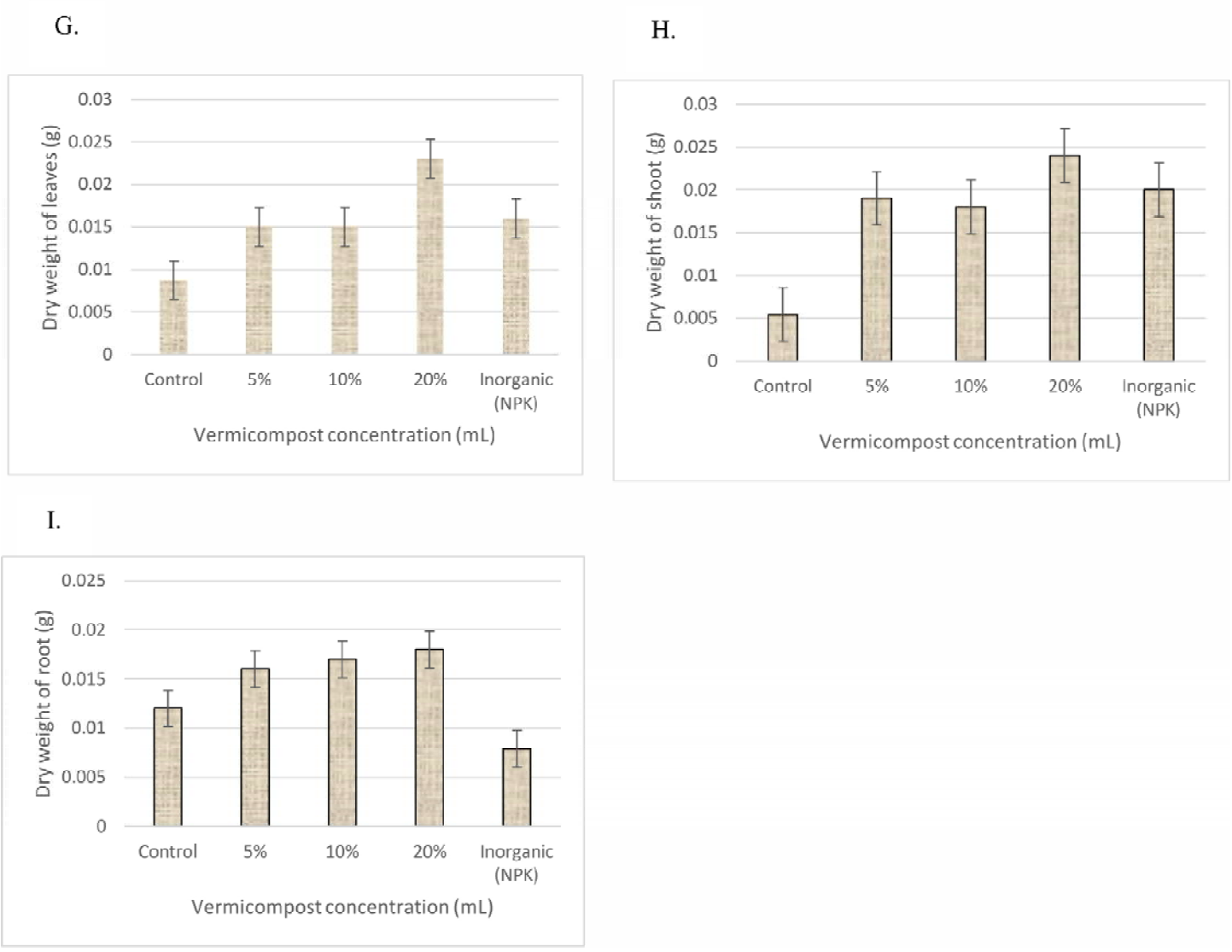

Figure 2.7: Maize germination responses of different vermicompost concentrations; A. Dry weight of leaves B. Dry weight of shoot C. Dry weight of root (cm) N = 5, mean ± S.E

inhibitors of the seed coat, the synthesis of late embryogenesis proteins which induced the mobilization and solubilization of globulins (Kaur *et al.*, 2018). This gives room for the expression of antioxidant enzymes. The malondialdehyde level induces α- amylase activity minimized during growth but largely improves plant performance and crop productivity (Kaur *et al.*, 2018). It ensures speedy and uniform germination. In this experiment pre-soaking for 10 hours seemed to have remarkably contributed to the germination and growth of seedlings which concurred with the results obtained by another study (Arancon *et al.*, 2008). These results acknowledge the stimulatory effect of pre-soaking and the application of vermicompost on germination and growth of plants. The positive influence of vermicompost treatment pre-soak on the morphological change of seedlings was evidence in previous findings, particularly in the growth of pea and tomato seedlings (Arancon *et al.*, 2008). The vermicompost extract produces polysaccharides which aggregate as cementing substances for better aeration, retention, drainage, aerobic condition for substrate and nutrient (Kaur *et al.*, 2018). It was also observed in a report that rapid germination was associated with seeds soaked in water before sowing (Wang *et al.*, 2002).

Sprouting was observed in all treatments between 24 and 72 hours after sowing. This involves treatment with pre-soaked in untreated control, vermicompost treated, and the inorganic fertilizer. Pre-soaking also, plays a significant role in determining the viability of seeds. The 10 per cent vermicompost concentration was found be effective for the germination of lettuce. This was probably because of the activity of the phosphatase in the earthworm gut and phosphorous solubilizing micro-organisms action in the worm casts of the earthworm under a mesophilic process. This supports the production of mucilage substances, growth hormones, nitrogenous discharge, and microbes during composting (Bhat *et al*., 2015; Atiyeh *et al*., 2000). The low vigor observed during germination may be due to the presence of phytotoxic and water-soluble bioactive substances such as humic acids, phytohormones, or the accumulation of microbial metabolites during vermicompost extract. Higher concentrations of vermicompost teas could limit the germination rates of seeds (Norman *et al*., 2012). A similar trend was found with the germination of tomato. This may be due to the presence of auxin, cytokinin, and gibberellin. It promotes plant height, root length, and density. The exception was the maize seedlings which behaved differently. The 20 per cent vermicompost concentration was found to be the most effective compared to the rest of the treatments. This may be due to the inhibitory action of the seed coat which induces the mobilization and solubilization of globulin which reduces the level of malondialdehyde production. The nutrients released from the activities of earthworms and microorganisms under the mesophilic condition was probably significant. The increases observed in root length with 5, 10 and 20 per cent vermicompost treatments may also be attributed to the presence of phytohormones as reported by (Govindapillai *et al*., 2018). However, the loss of seedlings was minimal after day twelve. This may be due to other factors such as light, temperature, moisture, and aeration.

Furthermore, higher germination rate was observed across all treatments. More than 50 per cent germination was observed in all the treatments, including inorganic treated seeds, except with tomato. These findings are similar to those observed by Chan *et al*., (2011) who indicated that a germination index higher than 50 per cent indicates a phytotoxin-free compost even though mortality was found across treatments. In other words, poor growth and germination may be due to prolong soaking (Vieira *et al*., 2004). Poor germination may also be related to poor aeration and low oxygen availability (Ali *et al*., 2016). The use of inorganic fertilizer shows that the use of inorganic fertilizer may reduce the osmotic potential of the seedlings. This may result in the accumulation of salt in the seeds (Esmaeilpour & Van Damme, 2016). Besides, it may also affect the microbial efficiency of the ecosystem. Inorganic fertilizer is not readily available to most farmers. Also, the minimum concentration of vermicompost should be used at germination. Increased concentrations may not necessarily add to morphological changes except seeds with large endosperms.

CONCLUSIONS

The results conclude that 10 per cent vermicompost concentration showed potential to increase the performance and growth of tomato and lettuce seeds. On the other hand, 20 per cent vermicompost treatment promoted the germination of maize. This indicates that seeds with large endosperm or epicarp may require a vermicompost leachate concentration between 10-20 per cent for germination. Concentrations higher than 20 per cent may be toxic. Inorganic fertilizer although resulted in 50 per cent germination but may not be advisable for seed germination because it might reduce seedling efficiency.

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Online Manuscript No. 98751 recived on 27.6.2022

Manuscript accepted for publication on 25.11.2022

Journal of Eco-friendly Agriculture **18**(1): 11-15. 2023
doi: 10.5958/2582-2683.2023.00002.3

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Effect of organic components and salt tolerant bacteria on growth parameters and physio-chemical properties of sodic soil on spinach

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ABSTRACT

A field experiment was conducted during 2018-19 at Mahtma Phule Krishi Vidyapeeth, Rahuri to study the effect of organic components and salt tolerant bacteria on growth parameters and physio-chemical properties of sodic soil on spinach. The results revelaed that the application of FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} in sodic soil recorded maximum plant height, fresh and dry weight of leaves (31.58 cm, 35.15 g, 3.54 g) of spinach plants which was at par with A_2 : urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (31.17 cm, 33.67 g, 3.39 g). The results of inoculation of salt tolerant bacteria in sodic soil revealed that consortia (*Pseudomonas* sp. + *Azotobacter* sp. + *Bacillus* sp.) recorded maximum plant height, fresh and dry weight of leaves (30.92 cm, 35.71 g, 3.42 g) and minimum was recorded in STB-2 : *Azotobacter* sp. (29.25 cm, 31.27 g, 3.27 g). The interaction of FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia recorded maximum plant height, fresh and dry weight of leaves than rest of the interaction treatments. The similar trend was reported in case of the physio-chemical properties of sodic soil 40 days after sowing. As regards the application of organic components in sodic soil, FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} significantly recorded maximum pH_s reduction (8.20) than rest of treatments as compared to initial pH_s (8.61) after 40 days of sowing. The results of inoculation of salt tolerant bacteria in sodic soil revealed that consortia (STB-1+STB-2+STB-3) recorded maximum reduction in pH_s (8.21) significantly superior than rest of treatments. The results of interaction of application of organic components and A_1 : FYM @ 5 t ha^{-1} + gypsum @ t ha^{-1} + consortia (8.11), A_2 = urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3 = *Bacillus* sp. (8.11) and 3) A_3 = Leaf litter compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1 = *Pseudomonas* sp. (8.11) recorded maximum pH_s reduction among the interactions. The pH_s reduction ranged from 8.11 to 8.40 among the interaction combinations as compared with initial pH_s (8.61). The organic carbon was ranged from 0.28 to 0.38 per cent among the interaction combinations. The slightly increase in organic carbon was due to application of organic components and inoculation of consortia reported in sodic soil as compared to initial organic carbon.

Keywords: Salt tolerant bacteria, Physio-chemical property, Organic component, Sodic soil, Spinach

Sodic soils have low infiltration rate, poor aeration and difficult to cultivate due to poor soil structure. Hence, plant growth adversely affected by sodic soils (Anonymous, 1998). Salt affected soil occupy 6.73 million of ha area (2.1% per cent of geographical area) of India out of which about 56 per cent are sodic and the remainder 44 per cent saline. The research study was conducted primarily in India has demonstrated useful effect of biological reclamation of sodic soil in particular to highly sodic and alkaline soil (More, 1994).

Larsen (1962) proposed four groups of microorganisms based on NaCl salt requirement as (i) non- halophilic grows best at less than 2 per cent salt, (ii) slight halophilic grows best at 2-5 per cent salt, (iii) moderate halophilic grows best a 5-20 per cent salt and (iv) extreme halophilic grows best at 20-30 per cent salt. Arora *et al.* (2016) isolated two halophilic bacteria from sodic soils from Indo-Gangetic plains of Uttar Pradesh. Salt removal efficiency of the strains were tested in liquid media. The inoculation of plant growth promoting halobacteria with wheat seed resulted in increase in grain (18.1%) and straw yield (24.2%) under sodic conditions. The soil pH of sodic soil reduced from 9.4 to 8.6 after application of halophilic bacteria consortia. Abed *et al*. (2004) reported that bacteria were very efficient in reducing the EC values in the first week of application by almost 50 per cent for all the soil samples. After that the reduction of EC was gradual and reached to almost 99.9 per cent after 104 days for some samples. The biotransformation of organic matter into organic acids and CO_2, can be attributed due to ameliorative effect of organic matter and an increase in microbial biomass and soil enzyme activities (Dick *et al*., 1988).

MATERIALS AND METHODS

The pot culture experiment was conducted in factorial completely randomized design with three replications during rabi season of 2018-2019 at M.P.K.V., Rahuri to see application of different organic components with inoculation of salt

tolerant bacteria on growth parameters of spinach crop. The used sodic soil was having pH_s 8.61, EC_e 3.12 dSm^{-1}, organic carbon (0.28%) and ESP (15.99%). The soil was steam sterilized at 1.1 kg/cm^3 pressure for 2 hrs for three successive days with interminent incubation. The earthen pots of size 30 cm diameter were surface disinfected and were filled with sodic soil @ 5.0 kg pot^{-1}. The different organic components factor A *viz.,* FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_1), urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_2), leaf litter compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_3), green leaf manuring (Glyricidia) @ 5 t h^{-1} + gypsum @ 5 t ha^{-1} (A_4) and factor B *viz.,* STB-1 : *Pseudomonas* sp., STB-2 : *Azotobacter*, STB-3: *Bacillus* sp. and 4. Consortia (STB-1 + STB-2 + STB-3). The Salt tolerant bacteria were isolated from sodic soil collected from D block of Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri during 2018-2019. The organic components (FYM, Urban compost, Leaf litter compost and Green leaf manure) were applied as per the treatments in sodic soil. The pots containing sodic soil was kept for incubation for 30 days. The incubation period was required for exchange of Na from clay complex by chemical amendments like gypsum and organic amendments for its biological degradation which helped for easily replacement of Na from clay complex. The inoculation of STB *viz.,* STB-1, STB-2, STB-3, and Consortia @ 1×10^{-9} cfu ml^{-1} was applied @ 20 ml pot^{-1} containing organic components according to treatments and kept for incubation for 30 days. The observations on growth parameters *viz.,* plant height, number of leaves, fresh and dry weight were recorded as well as physio-chemical properties *viz.*, pH_s, EC_e, organic carbon and ESP was analysed after 40 days after sowing of seeds of spinach. The data were subjected analysis for of critical differences as per the methods described by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Growth parameters of spinach

Height

The results on plant height (Table 1) revealed that the application of organic components in sodic soil, A_1: FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} recorded maximum height (31.58 cm) of plants which was at par with the treatment A_2 = urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (31.17 cm) after 40 days of sowing. The results of inoculation of salt tolerant bacteria in sodic soil revealed that consortia (STB-1+STB-2+STB-3) recorded maximum plant height (30.92 cm) and minimum was recorded in STB-2 = *Azotobacter* sp. (29.25 cm). The interaction *viz.*, application of FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + inoculation of consortia (*Pseudomonas* sp. + *Azatobacter* sp. + *Bacillus* sp.) recorded maximum plant height (33.67 cm) among the interaction treatments. Application of mixture of halotolerant bacteria significantly increased plant height, fresh weight and dry weight of mangrove seedling under salt stressed condition (Bashan *et al*. 1998). There was increase in plant height due to application of organic amendments and/or inoculation of halobacteria reported in sodic soil (Arora *et al.* 2016). The results revealed that the isolate DBS-42 and ASW-55 seems to be promising to have beneficial effect on the plant growth promoting parameters on tomato cv. Dhanshree in saline sodic soils (Gaikwad, 2015).

Fresh and dry weight of leaves

The results of fresh and dry weight of leaves of spinach at 40 days (Table 1) revealed that the application of organic components in sodic soil, the A_1 = FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} recorded maximum fresh weight and dry weight (35.15 g, 3.54 g) of plants which was at par with the treatment A_2 = urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (33.67, 3.39) after 40 days of sowing. The results of inoculation of salt tolerant bacteria in sodic soil revealed that treatment consortia (STB-1+STB-2+STB-3) recorded maximum fresh and dry weight of leaves (35.71 g, 3.42 g) which was followed by treatment STB-1 = *Pseudomonas* sp. (34.17 g, 3.36 g). The interaction *viz.*, application of FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + inoculation of consortia (*Pseudomonas* sp. + *Azatobacter* sp. + *Bacillus* sp.) recorded maximum fresh and dry weight (38.03 g, 3.82 g) among the interaction treatments. Bashan *et al*. (1998) reported that application of mixture of halotolerant bacteria significantly increased plant height, fresh weight and dry weight of mangrove seedling under salt stressed condition. Zahir *et al.* (2009) reported that the pea inoculated with *P. fluorescens* also found increase (45%) in growth parameters *viz.*, root and shoot length, fresh weight and number of leaves per plant of pea under salt stress. Different *Bacillus* sp. isolated and their seed inoculation with radish crop also found positively affected growth parameters such as fresh and dry weight of shoot and root in saline condition (Yildrim *et al*. 2008). The inoculation of DBS-42 and ASW-55 increased the maximum dry matter weight. The per cent increase in dry matter weight of tomato cv. Dhanshree to the tune of 38.30 to 45.14 per cent over control in saline sodic soils (Gaikwad, 2015).

Physio-chemical properties of sodic soil at harvesting

Changes in soil of physio-chemical properties *viz.,* pH, EC and ESP of spinach at 40 days as influenced by different treatments are presented in Table 2.

pH_s of sodic soil at harvest

The results (Table 2) revealed that the application of

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00002.3

Table 1. Effect of application of salt tolerant bacteria and organic components on growth parameters of spinach

Treatments	Height (cm)	Number of leaves (No.)	Fresh weighgt of leaves (g)	Dry weight of leaves (g)
Factor A				
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_1)	31.58	6.75	35.15	3.54
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_2)	31.17	6.58	33.67	3.39
Leaf litter compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_3)	30.17	6.25	31.68	3.18
Green leaf manuring (glyricidia) @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_4)	28.00	6.33	32.86	3.29
Mean	30.23	6.48	33.34	3.35
SE ±	0.08	0.30	0.26	0.03
CD at 5%	0.22	NS	0.76	0.08
Factor B				
Pseudomonas sp. (STB-1)	30.83	6.58	34.17	3.36
Azotobacter sp. (STB-2)	29.25	6.17	31.27	3.27
Bacillus sp. (STB-3)	29.92	6.25	32.18	3.35
Consortia (STB-1+ STB-2+ STB-3)	30.92	6.92	35.71	3.42
Mean	30.23	6.48	33.34	3.35
SE ±	0.08	0.30	0.26	0.03
CD at 5%	0.22	NS	0.76	0.08
Interaction A x B				
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1	31.67	7.33	34.67	3.48
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-2	30.00	6.00	33.43	3.37
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3	31.00	5.67	34.47	3.48
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia	33.67	8.00	38.03	3.82
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1	30.00	6.00	38.03	3.37
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-2	31.00	6.33	28.73	3.48
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3	33.67	7.67	34.47	3.82
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia	30.00	6.33	33.43	2.88
Leaf litter component @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1	33.67	7.67	28.73	3.82
Leaf litter component @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-2	30.00	6.33	30.67	2.88
Leaf litter component @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3	28.00	5.33	29.27	3.08
Leaf litter component @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia	29.00	5.67	38.03	2.93
Green leaf manuring (glyricidia) 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1	28.00	5.33	35.28	3.23
Green leaf manuring (glyricidia) 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-2	26.00	6.00	32.27	3.34
Green leaf manuring (glyricidia) 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3	27.00	6.33	30.53	3.06
Green leaf manuring (glyricidia) 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia	31.00	7.67	33.37	3.54
SE ±	0.20	0.60	0.51	0.05
CD at 5%	0.50	NS	1.51	0.15
Control for comparison	25.00	5.00	24.80	2.57

organic components in sodic soil, the treatment A_1 = FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} significantly recorded maximum pH_s reduction (8.20) than rest of treatments as compared to initial pH_s (8.61). The results revealed that consortia (STB-1+STB-2+STB-3) recorded maximum reduction in pH_s (8.21) significantly superior than rest of treatments. The results of interaction of application of organic components and inoculation of halobacteria i.e. A_1 = FYM @ 5 t ha^{-1} + gypsum @ t ha^{-1} + consortia (8.11), A_2 = urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3 = *Bacillus* sp. (8.11) and 3) A_3 = leaf litter compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1 = *Pseudomonas* sp. (8.11) recorded maximum pH_s reduction among the interactions. It was observed that the pH_s reduction was ranged from 8.11 to 8.40 among the interaction combinations as compared with initial pH_s (8.61). The reduction in pH_s due to application of organic components along with salt tolerant bacteria might be due to secretion of different organic acids by salt tolerant bacteria might lowered the pH_s of sodic soil. Similar results were reported (Gaikwad, 2015) and the consortia of halobacteria was inoculated and there was reduction in soil pH 9.4 to 8.6 in sodic aoils (Arora *et al.* 2016).

EC_e of sodic soil at harvest

The results (Table 2) revealed that the application of organic components in sodic soil, A_1 = FYM @ 5 t ha^{-1} +

Table 2. Effect of application of salt tolerant bacteria and organic components on physio-chemical parameters of spinach

Treatments	pH_s	EC_e $dS^{-1}m$	ESP (%)	OC (%)
Factor A				
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_1)	8.20	2.42	12.04	0.36
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_2)	8.22	2.44	12.20	0.34
Leaf litter compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_3)	8.24	2.49	12.47	0.32
Green leaf manuring (glyricidia) @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} (A_4)	8.33	2.66	13.19	0.31
Mean	8.25	2.50	12.48	0.33
SE ±	0.004	0.004	0.05	0.005
CD at 5%	0.01	0.01	0.15	0.02
Factor B				
Pseudomonas sp. (STB-1)	8.23	2.49	12.42	0.34
Azotobacter sp. (STB-2)	8.29	2.56	12.79	0.31
Bacillus sp. (STB-3)	8.26	2.52	12.50	0.33
Consortia (STB-1+ STB-2+ STB-3)	8.21	2.44	12.20	0.34
Mean	8.25	2.50	12.48	0.33
SE ±	0.004	0.004	0.05	0.005
CD at 5%	0.01	0.01	0.15	0.02
Interaction A x B				
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1	8.21	2.46	12.09	0.36
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-2	8.25	2.51	12.41	0.33
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3	8.24	2.48	12.22	0.35
FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia	8.11	2.22	11.43	0.38
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1	8.25	2.51	12.41	0.33
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-2	8.24	2.48	12.22	0.35
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3	8.11	2.22	11.43	0.38
Urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia	8.27	2.55	12.73	0.29
Leaf litter component @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1	8.11	2.22	11.43	0.38
Leaf litter component @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-2	8.27	2.55	12.73	0.29
Leaf litter component @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3	8.30	2.61	12.69	0.28
Leaf litter component @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia	8.28	2.57	13.01	0.33
Green leaf manuring (glyricidia) 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1	8.37	2.79	13.72	0.30
Green leaf manuring (glyricidia) 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-2	8.40	2.72	13.80	0.28
Green leaf manuring (glyricidia) 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3	8.38	2.75	13.64	0.30
Green leaf manuring (glyricidia) 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia	8.18	2.40	11.61	0.35
SE ±	0.007	0.008	0.10	0,01
CD at 5%	0.02	0.02	0.28	0.03
Control for comparison	8.61	3.12	15.99	0.28

gypsum @ 5 t ha^{-1} significantly recorded maximum EC_e reduction (2.42 dSm^{-1}) than rest of treatments as compared to initial EC_e (3.12 dSm^{-1}) after 40 days of sowing. The results of inoculation of salt tolerant bacteria in Sodic soil revealed that consortia (STB-1+STB-2+STB-3) recorded maximum reduction in EC_e (2.44 dSm^{-1}) significantly superior than rest of treatments. The results of interaction of application of organic components and inoculation of halobacteria i.e. A_1 = FYM @ 5 t ha^{-1} + gypsum @ t ha^{-1} + consortia, A_2 = urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3 = *Bacillus* sp. and 3) A_3 = leaf litter compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1 = *Pseudomonas* sp. recorded maximum reduction in EC (2.22 dSm^{-1}) among the interactions. It was observed that the EC_e reduction was ranged from 2.22 to 2.79 dSm^{-1} among the interaction combinations as compared with initial EC_e (3.12 dSm^{-1}). Similar results were reported by (Gaikwad, 2015) and (Arora *et al*. 2016) that an increase in EC_e reduction was due to application of organic components and/or inoculation of halobacteria reported in sodic soil as compared to initial EC_e.

ESP of sodic soil at harvest

The results (Table 2) revealed that the application of organic components in sodic soil, A_1 = FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} significantly recorded maximum ESP reduction (12.04%) than rest of treatments as compared to initial ESP (15.99%). The results of inoculation of salt tolerant bacteria in sodic soil revealed that consortia (STB-1+STB-

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00002.3

2+STB-3) recorded maximum reduction in ESP (12.20%) significantly superior than rest of treatments. The results of interaction of application of organic components and inoculation of halobacteria i.e. A_1 = FYM @ 5 t ha^{-1} + gypsum @ t ha^{-1} + consortia (11.43%), A_2 = urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3 = *Bacillus* sp. (11.43%) and 3) A_3 = leaf litter compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1 = *Pseudomonas* sp. (11.43%) recorded maximum ESP reduction among the interactions. It was observed that the ESP reduction was ranged from 11.43 to 13.80% among the interaction combinations as compared with initial ESP (15.99%). The reduction in ESP was due to application of organic components and inoculation of salt tolerant bacteria reported in Sodic soil as compared to initial ESP. Similar results were recorded by (Arora *et al*. 2016).

Organic carbon (%)

The results (Table 2) reaveled that the application of organic components in Sodic soil, the treatment A_1 = FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} recorded maximum organic carbon (0.36%) as compared to initial value 0.28%. The results of inoculation of salt tolerant bacteria in sodic soil reported that consortia (STB-1+STB-2+STB-3) and STB-1 = *Pseudomonas* sp. recorded maximum organic carbon (0.34%) and minimum organic carbon was recorded by STB-2 = *Azotobacter* sp. (0.31%). The results of interaction of application of organic amendments and inoculation of salt tolerant bacteria revealed that, the treatment combination i.e. A_1 = FYM @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + consortia, A_2 = urban compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-3 i.e. *Bacillus* sp. and A_3 = leaf litter compost @ 5 t ha^{-1} + gypsum @ 5 t ha^{-1} + STB-1 i.e. *Pseudomonas* sp. recorded maximum organic carbon (0.38%) among the interactions. The organic carbon was ranged from 0.28 to 0.38 per cent among the interaction combinations. The slightly increase in organic carbon was due to application of organic components and inoculation of salt tolerant bacteria reported in sodic soil as compared to initial organic carbon.

CONCLUSION

It can be concluded that application of salt tolerant bacterial consortia alongwith organic components could increase plant height and dry matter weight of spinach crop reduce in pH, EC and ESP and slightly increase in organic carbon of sodic soils.

ACKNOWLEDGEMENTS

The authors are thankful to the Head, Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri for providing the facilities for the conduct of present investigation.

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Online Manuscript No. 99080 recived on 17.7.2022
Manuscript accepted for publication on 11.11.2022

Journal of Eco-friendly Agriculture **18**(1): 16-19. 2023
doi: 10.5958/2582-2683.2023.00003.5

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Assessment of the effect of organic manure and biofertilizers on physiological attributes of cherry tomato genotypes

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ABSTRACT

An experiment was undertaken to assess the effect of three levels of vermicompost doses and the biofertilizer on the physiological attributes of five genotypes of cherry tomato during the *Rabi* season in 2018-19 under open field condition. The physiological traits were observed significantly affected by organic manure and biofertilizer. The genotype 2018/TOC R-1 with leaf area index (2.29) and leaf area diameter 248103.9 cm^2 × days) was found slightly better than variety G4. 2018/TOC VAR-1 registered highest light transmission ratio (73.40%). As regards to physiological parameters, recorded the maximum, application of vermicompost 5 t ha^{-1} and *Azotobacter* 4 kg ha^{-1} chlorophyll content index (47.85), LTR value (69.04%), LAI (2.13) and lowest Ei (0.40 $calcm^{-2}min^{-1}$). 2018/TOC VAR-1 under recommended dose of fertilizer gave the lowest magnitude of LTR (78.77%) and highest energy interception (0.61 $calcm^{-2}min^{-1}$). Maximum LAI (2.41) and LAD (260603.7 cm^2×day) was recorded in 2018/TOC VAR-6 grown with vermicompost (5 t ha^{-1}) and *Azotobacter* (T15).

Keywords: Cherry tomato, Physiological traits, Vermicompost, LAI, LAD, LTR, EI.

Cherry tomato (*L. esculentum* cv. *cerasiforme*), indigenous to Peru-Ecuador, is an ancestor of the modern tomatoes grown round the globe. The physiology of the cherry tomato plant is similar to those of the tomato plants possessing determinate, semi-determinate and indeterminate growth habit having long raceme and small, intense coloured and flavoured fruits weighing 10-30 g (Prema *et al*., 2011). The application of forage fertilization through organic supplements such as vermicompost and biofertilizers helps in the physiological development of the cherry tomato plants till the moment of blooming and formation of leaf which contains higher chlorophyll content, nutrients and biomass content. Chlorophyll content in the leaves and its area using non-destructive portable devices help in determining and monitoring the nutritional state of the plant (Silveira *et al*., 2003).

The supply of essential macro and micronutrients using organic source, not only improves the efficacy of the soil but also enhances the growth and development of the plants by increasing the rate of photo-assimilation and helps in translocation of produce from source (root) to sink (leaf) (Palekar, 2006). The main objective to carry out the present study was to determine the effect of organic manure and biofertilizer on physiological attributes of cherry tomato genotypes.

MATERIALS AND METHODS

The experiment was carried out at Horticulture complex, Department of Horticulture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) during the year *Rabi* season in 2018-19. The experiment was arranged in a Factorial Randomized Completely Blocked Design (RCBD) with three replications comprising of two factors namely cherry tomato genotypes and varied doses of vermicompost with *Azotobacter*. The experiment consisted of fifteen treatment combination including five genotypes viz. 2018/TOC VAR-1, 2018/TOC VAR-2, 2018/TOC VAR-4, 2018/TOC VAR-5, 2018/TOC VAR-6, coded as genotypes G1, G2, G4, G5 and G6, respectively and two doses of vermicompost and one of *azotobacter* viz., control (RDF), vermicompost (2.5 t ha^{-1}) + *Azotobacter* (4 kg ha^{-1}) and vermicompost (5 t ha^{-1}) + *Azotobacter* (4 kg ha^{-1}).

The data on different physiological parameters viz., chlorophyll content index (CC1), light transmission ratio (LTR), energy interception (E1) leaf area index (LA1) and leaf area diameter (LAD) were statistically analysed.

RESULTS AND DISCUSSION

Chlorophyll content index (SPAD value)

Portable chlorophyll meter SPAD was a non-destructive, sensible and precise means to determine the

chlorophyll content of cherry tomato leaves to identify the nutritional state of the plant. The data pertaining to CCI is presented in table 1 and Fig. 1.

The chlorophyll content index (CCI) revealed significant difference among the factor I, factor II and their interactions. The genotype 2018/TOC VAR-6 was found to be associated with the highest chlorophyll content index (45.50). G2 (45.00) and G4 (45.12) were found to be at par with G6. However, the lowest chlorophyll index (42.1) was recorded in G1 2018/TOC VAR-1. Application of vermicompost (5 t ha^{-1}) and *Azotobacter* (4 kg ha^{-1}) (B3) was found to be associated with highest chlorophyll content index (47.85). While, B1 (100% RDF) possessed the lowest (39.91) value of chlorophyll index. Similar findings were observed by Arouiee *et al*. (2009), Mogle and Chamle (2011), Hassan *et al*. (2018) and Wang *et al*. (2017) which showed that the increase in the CCI was due to combined applications of biofertilizers with vermicompost which might provide readily available forms of essential elements into the soil. Ferreira (2006) evaluated the RCI and chlorophyll content in the leaf blade of the tomato plant concerning the doses of nitrogen and organic fertilization in two harvest time. Among interactions treatment T9 showed significantly highest magnitude of CCI (49.56). Leaf chlorophyll content is correlated with leaf nitrogen status, photosynthetic capacity and RuBP carboxylase activity (Evans, 1998).

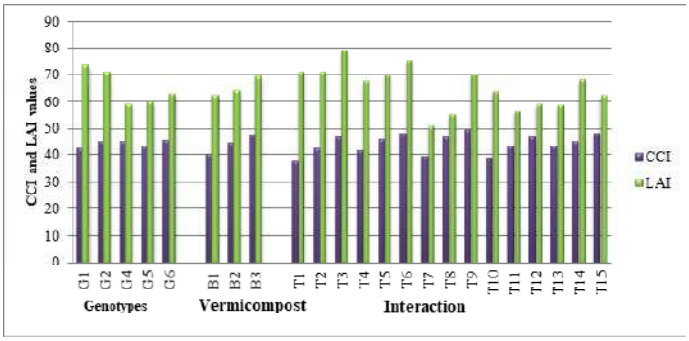

Fig. 1: Graphical representation of Physiological Parameters (CCI and LAI) of Cherry Tomato

Leaf area index (LAI)

Leaf area is contemplates in the physiological studies and crop characterization to determine growth, leaf area index etc. Leaf area index (LAI) is a dimensionless variable which illustrates the structural trait of leaf components, estimated by area of leaf per unit area of soil surface. LAI displays leaf photon interception which affects the biomass & productivity (Firouzabadi *et al*. 2015). It stimulates how much light moves through a canopy and influences the microclimate; thus, it can be used as an indicator of canopy health or development (Wang *et al*., 2017).

The genotypes and vermicompost doses significantly influenced the leaf area index (LAI). Maximum leaf area index (2.29) was recorded in genotype 2018/TOC VAR-6 and minimum (1.28) in genotype 12018/TOC VAR-1. Maximum leaf area index of (2.41) recorded under vermicompost (5 t ha^{-1}) with *Azotobacter* (B3) showed superiority over rest of the treatments. The lowest value (1.04) for this character was noted under T1 2018/TOC VAR-1 with control (NPK). These findings are in conformity with the findings of Azarmi *et al*. (2008) and Arouiee *et al*. (2009).

LAD (Leaf area diameter)

In the present study, maximum value (248103.9 cm^2 × days) for LAD was recorded in G6 (2018/TOC VAR-6) and

Table 1: Mean performance of physiological characters

Treatments	CCI	LTR (%)	EI (cal/cm^2/min)	LAI	LAD (cm^2 days)
Different Genotypes					
G1 2018/TOC VAR-1	42.10	73.48	0.35	1.28	1,44,493.9
G2 2018/TOC VAR-2	45.00	70.71	0.38	2.02	2,19,022.9
G4 2018/TOC VAR-4	45.12	58.79	0.52	1.99	2,08,225.0
G5 2018/TOC VAR-5	42.90	59.63	0.50	2.12	2,27,670.7
G6 2018/TOC VAR-6	45.50	62.94	0.47	2.29	2,48,103.9
S.Em±	0.45	1.20	0.019	0.05	6,191.36
C.D.5% level	1.32	3.49	0.055	0.15	18,028.21
Vermicompost Doses					
B1 Control	39.91	62.33	0.46	1.70	1,86,575.3
B2 Vermicompost 2.5 tha^{-1}	44.63	63.99	0.47	1.95	2,08,477.3
B3 Vermicompost 5 tha^{-1}	47.85	69.04	0.41	2.13	2,33,457.3
S.Em±	0.32	0.85	0.015	0.03	4,795.81
C.D.5% level	0.94	2.47	0.043	0.10	13,964.59
Interaction					
T1 G1B1	37.33	70.82	0.39	1.04	114169.67
T2 G1B2	42.28	70.84	0.36	1.20	135575.72
T3 G1B3	46.79	78.77	0.31	1.62	183736.4
T4 G2B1	41.22	67.56	0.41	1.87	197866.96
T5 G2B2	45.89	69.61	0.42	2.18	236383.83
T6 G2B3	47.91	74.98	0.31	2.01	222817.76
T7 G4B1	39.14	50.99	0.61	1.73	183419.78
T8 G4B2	46.67	55.24	0.53	1.91	188897.55
T9 G4B3	49.56	70.14	0.41	2.32	252357.74
T10 G5B1	38.85	63.60	0.42	1.98	211960.07
T11 G5B2	42.98	56.31	0.56	2.07	223281.23
T12 G5B3	46.88	59.00	0.51	2.29	247770.73
T13 G6B1	43.04	58.53	0.53	2.06	225459.83
T14 G6B2	45.33	67.99	0.41	2.39	258248.22
T15 G6B3	48.13	62.32	0.47	2.41	260603.73
S.Em±	0.79	2.09	0.033	0.09	10,723.75
C.D.5% level	2.30	6.05	0.096	0.26	31,225.77

minimum (144493.9 cm^2 × days) in G1 (2018/TOC VAR-1). Under vermicompost levels B3 (vermicompost 5 t ha^{-1} and *Azotobacter*) possessed the highest magnitude (233457.27 cm^2 × days) for this trait which exhibited significant impact over the rest. control recorded the lowest value (186575.26 cm^2 × days) for this character. The results were in accordance with the findings of Hassan *et al*. (2018) who observed maximum LAD at all stage with the combined application of inorganic fertilizer (RDF) and FYM @ 38 t ha^{-1}. LAD is a useful concept in depicting the photosynthesis system and finally leading to a most complex character i.e. yield. The treatment combinations exhibited significant difference for LAD. Treatment combination T15, 2018/TOC VAR-6 under 5 t ha^{-1} vermicompost and *Azotobacter* (260603.7 cm^2×day) was found significantly superior over the rest except T14 (258248.2 cm^2×day) which showed numerical differences with the former. Minimum LAD (114169.7 cm^2.day) was noted in T1 (2018/TOC VAR-1) with control (RDF). Higher LAD facilitates higher amount of production of photo assimilates. *Azotobacter* significantly increased LAI and LAD. It might be due to role of nitrogen enhancing persistence and longevity of LA, which is a key factor in terms of photosynthesis productivity of the plants, that assimilates higher amount of photosynthates production and if the mobilization is proper to the sink, it will enhance the economic productivity. The findings are supported by Peng *et al*. (2013).

Light transmission ratio (LTR)

The data pertaining to light transmission ratio (LTR), estimated using Luxmeter, showed that the treatments varied significantly with respect to LTR. Among the genotypes, G1 2018/TOC VAR-1 exhibited significantly more LTR (73.48%) over the rest of the genotypes, except G2 2018/TOC VAR-2 (70.71%) which was at par. G4 2018/TOC VAR-4 possessed the lowest magnitude (58.79%) for this trait though did not differ significantly with G5 (59.63%). In factor B, among the different doses of vermicompost, the B3 (vermicompost 5 t ha^{-1} along with *Azotobacter)* having LTR value of 69.04% significantly superseded other treatments. B1 control had the minimum LTR (62.33%). Among interactions, treatment combination the T3 2018/TOC VAR-1 and vermicompost (5 t ha^{-1}) with *Azotobacter* exhibited significant superior LTR (78.77%) over the rest, except T6 which showed numerical differences with the former which were non-significant. The findings of Thakur and Kaur, 2001 were in close conformity with present findings.

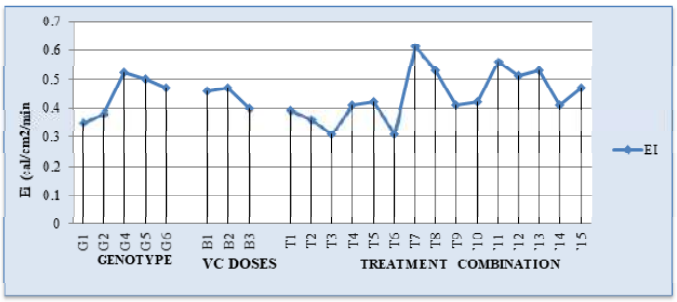

Fig. 2: Graphical representation of energy interception in Cherry tomato under different treatments

Energy interception

The data on energy interception (Ei) presented in table 1 and fig. 2 showed that all the factors and their interactions deferred significantly at an interval of 90 days after transplanting. High significant variation existed among factor A, G4 2018/TOC VAR-4 indicated maximum EI (0.52 cal/cm^2/min) though was at par with G5 2018/TOC VAR-5 and G6 2018/TOC VAR-6 having EI 0.50 and 0.38 cal/cm^2/min respectively. It may be due to scattered canopy at different canopy layers which might have produced the shading effect to the lower canopy layers which might have resulted in reduced energy interception. Among factor B, B1 control (RDF) with Ei 0.47 cal/cm^2/min had the maximum magnitude though it did not indicate any significant difference with B2 (0.46 cal/cm^2/min). B3 recorded minimum E1 (0.41 cal/cm^2/min).

The interaction of genotype with different doses of vermicompost showed significant effect on Ei. T7 2018/TOC VAR-4 Control (RDF) possessed significantly more EI (0.61 $calcm^{-2}min^{-1}$) over the rest except T11 (0.56 cal/cm^2/min) and T13 (0.53 $calcm^{-2}min^{-1}$) which were at par. Treatment combinations T3 and T6 possessed minimum (0.31 $calcm^{-2}min^{-1}$) EI. The probable reason may be that the interception of light by a crop canopy is strongly related to total leaf area and orientation. Crop will thus intercept more PAR and hence grow faster if it develops leaf area rapidly.

CONCLUSION

The physiological parameters showed significant variation thus giving different results for different treatments. Chlorophyll content index (49.56) was found to be maximum in T9. 2018/TOC VAR-1 under recommended dose of fertilizer (T7) gave the lowest magnitude (78.77%) of LTR and highest Ei (0.61 $calcm^{-2}min^{-1}$). Maximum LAI (2.41) and LAD (260603.7 cm^2×day) was recorded in 2018/TOC VAR-6 grown with vermicompost (5 t ha^{-1}) and *Azotobacter* (T15).

ACKNOWLEDGEMENT

The author wish to thank the research guide, Head and staff of the Department of Horticulture and Dept. of Plant

Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh for the assistance, their suggestions, untiring help and moral support they provided during the course of the study.

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Online Manuscript No. 98353 recived on 29.5.2022
Manuscript accepted for publication on 11.11.2022

Journal of Eco-friendly Agriculture **18**(1): 20-26. 2023
doi: 10.5958/2582-2683.2023.00004.7

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Screening of nitrogen fixers from root nodules associated with *Albizia procera*

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ABSTRACT

The rhizosphere contains a host of microorganisms that are affected by both abiotic stress and biotic stress. Low fertility is a major problem in establishing vegetation in degraded areas. Nitrogen (N) is an important plant nutrient needed for plant growth in abundance in the earth's atmosphere however; most tropical soils are deficient in available N. In low-lying ecosystems and without human fertilization or supplementation, the nutrients found in plants come from organic matter or organic matter. The amount of fixed nitrogen depends on the nitrogen level of the soil, the type of rhizobia that attacks the pods, the growth of the plant and the length of the growing season. Nitrogen-fixing tree species can play an important role in stabilizing sandy and eroded soils, utilizing deep groundwater due to its broad roots and thus helping to increase soil fertility. Among the indigenous legume species Albizia procera is best known for the traditional legume species, which are associated with Rhizobium and this association leads to nitrogen fixation in the atmosphere.

Soil rhizobial value calculations showed high number of bacteria in the soil sample of Baddi (297.21 cfu g^{-1}) and a small amount in Solan (243.46 cfu g^{-1}). On the basis of verification tests namely, congo red test, ketolactose testing and plant infection testing in all isolated areas, more than 70.58 per cent (26 of 34) were confirmed as rhizoba. All isolates are tested for factors that promote the growth of different plants, namely, p-solubilizers, siderophore producers and IAA producers. Of these 26 rhizobial isolate, 82.38 per cent were p-solubilizers, 54.45 per cent were siderophore producers and 75.47 per cent were IAA producers. This approach was to improve the bio-inoculum to increase soil fertility by injecting the most suitable type of bacteria that would bring the best plant into the natural environment without adding fertilizer from the outside. Rhizobia is very important in improving the availability of nitrogen in the soil.

Keywords: *Albizia procera,* Rhizobia, P-solubilizers, siderophore, IAA

Albizia procera, commonly known as 'Safe Siris', is one of the important species of nitrogen fixing tree (NFT), belonging to Fabaceae, sub-family Mimoscidae. It is a large, fast growing medium to large sized deciduous tree that occurs at many different sites. It is distributed through moist and dry deciduous forests of India. This species provides wood for various purposes, nutritious fodder for livestock and shade for tea gardens. It is extensively planted in agricultural land, barren land, roadside paths and is an important afforestation and agro-forestry species. It provides an excellent fuel, fodder and small wood for making agricultural equipment (Singh, 1982). In addition, it is an important nitrogen fixing tree and helps in increasing the productivity of the soil. The wood is commonly employed in making material for carts, carts, small handle tools and agricultural implements and also provides fodder during short periods of summer, when there is a shortage of green fodder in the region.

Natural regeneration of *Albizia procera* is often encouraged by providing small wood, fuel wood, charcoal, fodder or shade on farms. Keeping this in view an attempt to examine plant growth promoting traits of rhizobia isolated from *Albizia procera* was made with the following objectives:

(i) Isolation and certification of rhizobial isolates from root nodules of Albizia procera.

(ii) Screening of rhizobial isolates for various plant growth promoting traits.

MATERIAL AND METHODS

The present investigation was conducted to explore various plant growth promoting traits of rhizobia isolated from *Albizia procera* root nodules. Samples of *Albizia procera* root nodules were collected from 5 different locations in Himachal Pradesh. viz, UHF Nauni, RHR&TS Jachch, Gaggal, RHR&TS Dhaulakuan and Baddi. Yeast extract was

isolated from root nodules on mannitol agar medium. They were further subjected to multiple certification tests. Congo red test (Vincent, 1970), bromothymol blue test (Vincent, 1970), Hofer's growth in alkaline broth (Hofer, 1935), ketolactose medium (Bernarts and Daly, 1963) and plant infection test (Vincent, 1970). After certification of rhizobial isolates, these isolates were assessed for various plant growth-promoting traits, such as, P-solubilization (Pikovskaya, 1948), siderophore production (Schwinn and Nielands, 1987) and IAA production (Pikovskaya, 1948), Vincent, 1947).

RESULTS AND DISCUSSION

Isolation of Rhizobia from root bodies of different seed sources of *Albizia procera* seedlings

A total of 38 rhizobial strains were isolated from root nodules of *Albizia procera* collected from various sites in Himachal Pradesh. Of the 38 all isolates screened for various certification tests, 27 showed positive results for all certification tests (Table 1).

The variation in the rhizobial population colonizing the roots of *Albizia procera* seedlings is presented in Table 1 showed the maximum (86.4 × 102 cfu g^{-1} root) rhizobial count on Yema medium at the Una site and the minimum (55.6 × 102 cfu g^{-1} root) at Haridwar. Variation in rhizobia populations can be attributed to the influence of root exudates, plant age, soil biogeochemical and physico-chemical properties, and environmental conditions (Wieland *et al.*, 2011). Balota and Chaves (2011) and Zhou *et al.* 2017 also reported the greatest variation in microbial populations with respect to legumes.

Authentication of rhizobia isolated from root nodules of Albizia procera plant

All the 38 isolates were accordingly assigned different codes to each isolate. These were screened for various certification tests such as, congo red test, bromothymol blue test, growth in Hofer's alkaline broth, ketolactose medium, glucose peptone agar and plant infection test (Table 2 and Plate 1). 22 out of 38 isolates gave positive results for all certification tests. Thirty isolates were unable to absorb the congo red dye on cryoma medium after an incubation period for 3–5 days at 28 ± 2 °C. Twenty-eight isolates showed moist and viscous colonies and the surrounding medium was yellow because of acid production on YEMA medium enriched with bromothymol and after incubation for 3–5 days at 28 ± 2 °C. None of the isolates with pH 11.0 were able to grow in Hofer's alkaline broth. Lactose and peptone were not used in any of the isolates. Plant infection tests revealed that only 26 out of 38 isolates nodulated *Albizia* seedlings at 75 days post inoculation and these isolates were provisionally confirmed as *Rhizobium* spp.

Table 1. Isolation of rhizobia from root nodules of *Albizia procera*

	Sites	Isolate name	No. of isolates	Rhizobial population (10^2 cfu g^{-1} soil)
Himachal Pradesh	**Nauni**	NA (Nauni *Albizia*)	8	80.3
	Baddi	BA(Baddi *Albizia*)	10	65.6
	Gaggal	GA(Gaggal *Albizia*)	6	58.2
	Jachh	JA(Jachh *Albizia*)	9	71.3
	Dhaulakaun	DA(Dhaulakaun *Albizia*)	5	86.4
CD (0.05%)				4.34

Plate 1. Authentication test by rhizobial isolates a) growth on YEMA medium, b) congo red test, c) bromothymol blue test, d) ketolactose test and e) plant infection test

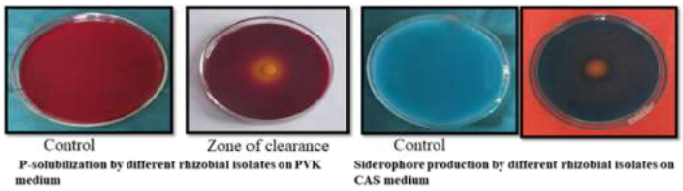

Plate 2: Phosphorus solubilization, siderophore and IAA production by rhizobial isolates

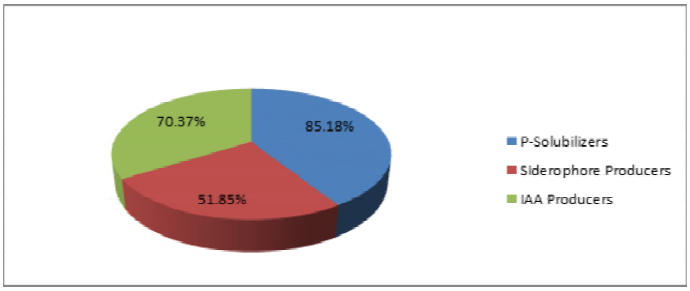

Fig. 1: Percentage of Phosphorus solubilization, Siderophore and IAA production by rhizobial isolates.

Table 2. Various authentication tests by rhizobial isolates

Rhizobial isolates	Congo red test	Bromothymol blue test	Ketolactose test	Growth in Hofer's alkaline broth	Infectivity test (Nodule formation)
NA1	-	Alkaline producer	-	No growth	Present
NA2	-	Alkaline producer	-	"	Present
NA3	-	Alkaline producer	-	"	Present
NA4	-	Alkaline producer	-	"	Present
NA5	-	-	-	"	-
NA6	-	Alkaline producer	-	"	-
NA7	-	Alkaline producer	-	"	Present
NA8	-	-	-	"	-
BA1	-	Alkaline producer	-	"	-
BA2	-	Alkaline producer	-	"	Present
BA3	-	Alkaline producer	-	"	-
BA4	-	Alkaline producer	-	"	Present
BA5	-	Alkaline producer	-	"	Present
BA6	-	-	-	"	-
BA7	-	Alkaline producer	-	"	-
BA8				"	
BA9	-	Alkaline producer	-	"	Present
BA10				"	
JA1	-	Alkaline producer	-	"	-
JA2	-	Alkaline producer	-	"	Present
JA3				"	
JA4	-	Alkaline producer	-	"	Present
JA5	-	Alkaline producer	-	"	-
JA6	-	Alkaline producer	-	"	Present
JA7				"	
JA8				"	
JA9	-	Alkaline producer	-	"	-
DA1	-	Alkaline producer	-	"	Present
DA2	-	Alkaline producer	-	"	Present
DA3	-	Alkaline producer	-	"	-
DA4				"	
DA5	-	Alkaline producer	-	"	Present
GA1	-	Alkaline producer	-	"	Present
GA2	-	Alkaline producer	-	"	-
GA3	-	Alkaline producer	-	"	Present
GA4	-	-	-	"	-
GA5	-	Alkaline producer	-	"	Present
GA6	-	-	-	"	-

- Negative, + Positive, Acid producer: Fast growing, Alkali producer: Slow growing,

The results of the present investigation are in confirmation of the findings of Agarwal *et al.* (2012) who also reported that Rhizobial isolates did not absorb red dye when ingested in yama containing congo Red dye and did not grow in Hofer's alkaline broth. They also did not use lactose and peptone. The results were further supported by Deshwal and Choubey (2014) and Dipta *et al.* (2017).

Screening of rhizobial isolates for diverse plant growth promoting traits

Twenty seven isolates from HP, after certification tests, were further purified on YEMA medium. These were examined for p-solubilization, siderophore, IAA and HCN production and against *Fusarium oxysporum, Fusarium graminearum, Pythium ephenidermatum, Alternaria, Rhizoctonia solani* and *Colletotrichum capsici*. Of these 85.18 per cent were

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00004.7

p-solubilizers, 51.85 per cent were siderophore producers and 70.37 per cent were IAA producers and only 10.52 per cent were found to produce HCN (Figure 1). Rhizobial isolate BA2 displayed maximal plant growth promotion (PGP) characteristics.

Quantification of plant growth promoting traits of selected rhizobial isolates

The selected 27 isolates were further subjected to quantitative assessments of tricalcium phosphate, siderophore production and indole 3 acetic acid production. The data presented in Table 3 showed that the HP isolates exhibited a maximum (165.0 µg ml^{-1}) P solubility with the BA2 isolate with a decrease in pH from 7.0 to 3.4. Whereas, isolate the GA5 soluble minimum (33.67 µg ml^{-1}) TCP in liquid medium with a decrease in final pH from 7.0 to 5.04.

Phosphorus (P) is the second most essential macronutrient for high and sustained agricultural productivity after nitrogen and accounts for about 0.2–0.8 percent of the total dry weight (Goswami *et al.,* 2014). The group of microorganisms capable of converting insoluble P into soluble is collectively called phosphate soluble bacteria. They increase the availability of phosphorus to the plant by lowering soil pH through organic acid chelation, production of ion exchange reactions (Delvasto *et al.* 2006) and mineralization of organic P by phosphatases and lysates (Sharma *et al.* 2013). The results of the present study are in confirmation with those of Sridevi and Mallaiah (2009), Kumar and Ram (2014) and Rafaki *et al.* (2015) who reported P-solution by Rhizobium species. Anton *et al.* (1998) reported that out of 266 rhizobial strains, 54 percent of the P-solubilizers were Rangel *et al.* (2017) reported 89 percent of the 19 isolates as P solubilizers rhizobia associated with Leucaena leucocephala.

The siderophore production capacity of the selected isolates was confirmed using the Chrome Azuroles (CAS)

Table 3. Quantification of PGP traits by selected rhizobial isolates

Isolates	P-solubilization		Siderophore production		IAA production	
	P-solubilization (µg/ml)*	Final pH of supernatant	Siderophore estimation (% SU)**	Final pH of supernatant	IAA production (µg ml^{-1})	Final pH of supernatant
NA1	90.00	4.72	25.03	6.25	59.18	5.75
NA2	67.17	5.37	8.98	6.70	38.71	6.20
NA3	ND	ND	ND	ND	42.67	5.94
NA4	125.00	4.43	ND	ND	ND	ND
NA5	84.46	4.81	15.94	6.43	17.50	6.40
NA6	ND	ND	ND	ND	ND	ND
NA8	100.00	4.55	37.78	6.18	42.00	5.98
BA1	ND	ND	7.29	6.65	38.00	5.10
BA2	165.00	3.40	63.23	4.70	86.00	4.80
BA3	35.67	5.04	32.75	6.20	71.00	5.12
BA5	74.23	5.23	60.36	6.32	ND	ND
BA7	ND	ND	ND	ND	62.00	5.45
BA8	65.44	5.54	45.77	ND	ND	ND
BA10	40.00	5.76	23.22	ND	ND	ND
JA1	ND	ND	25.65	6.27	49.00	6.30
JA2	50.00	5.69	17.65	6.37	ND	ND
JA3	45.77	5.34	5.88	5.37	35.00	5.45
JA5	ND	ND	ND	ND	34.00	5.50
JA7	75.00	3.40	65.23	4.70	76.00	4.80
JA9	ND	0.12	6.27	0.25	54.56	0.19
DA1	ND	ND	21.50	6.70	ND	ND
DA3	115.00	4.98	38.61	6.70	70.00	5.70
DA5	ND	ND	ND	ND	35.00	5.75
GA2	105.00	5.30	48.92	6.10	ND	ND
GA4	ND	ND	ND	ND	29.00	5.25
GA5	33.67	5.04	34	ND	11.00	5.38
GA6	135.00	4.12	28.50	6.40	36.00	5.34
CD(0.05%)	7.23	0.12	5.67	0.29	4.78	0.21

ND- Not detected

assay. Among the 27 isolates from HP, the maximum (63.23%) siderophore production potential was recorded with BA2, with a decrease from pH 7.0 to 4.70, with BA5 (60.36%). Minimal (5.88%) siderophore production efficiency was recorded for JA3 with a decrease in pH from 7.0 to 5.37 (Table 3). Siderophore production by microorganisms improves iron availability to plants (Kotasthene *et al.,* 2017). Microorganisms release siderophores to scavenge iron from the mineral phase by forming Fe3+ complexes. Siderophore producing microorganisms protect plants at two levels i.e. by limiting the growth of plant pathogens and by triggering the plant's defensive mechanisms (Ramos Solano *et al.,* 2010).

IAA production by the selected isolates varies between 11.50 and 86.00 µg ml^{-1} (Table 3). In HP isolates, BA2 produced a significantly higher amount of IAA, i.e. 86 µg ml^{-1}, reducing the final pH from 7.00 to 4.80, followed by DA3 (70.00 µg ml^{-1}) after 72 hours of incubation at 28 °C. After and having a minimum of IAA (11.50 µl mg^{-1}) was recorded with the GA5 isolate. The bacterium IAA stimulates the root development of the host plant resulting in better absorption of water and nutrients from the soil (Bandivadekar *et al.,* 2016). IAA affects plant cell division, expansion and differentiation, stimulating seed and tuber germination, increasing the rate of xylem and root development; controls the processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and inflorescences; Affects photosynthesis, pigment formation, biosynthesis of various metabolites and resistance to stressful conditions. Microbial synthesis of indole acetic acid (IAA) stimulates and facilitates plant growth (Mohite, 2013). The results of the present investigation are in line with those of Saraylu *et al.* (2014) who reported indole acetic acid (IAA) production in the range of 63.42 to 93.62 mg ml^{-1} by Rhizobium rhizogene isolated from soybean. Ghosh *et al.* (2015) reported IAA production from nodules of the aquatic legume Neptunia oleracea ranging from 148.0 µg/mL to 226.0 µg ml^{-1}.

Evaluation of selected rhizobial isolates for antifungal activities

Rhizobial isolates displayed significant variation in antifungal activities against the tested pathogens (Table 4). The maximum inhibition (27.43%) was recorded for isolate BA2 for *F. graminearum, P. ephenidermatum* (25.67%), *Alternaria* (35.71%), *R. soloni* (37.07%) in isolates from HP.

Table 4. Antifungal activity against various pathogens

Isolate	*Fusarium oxysporum*	*Fusarium graminearum*	*Pythium aphanidermatum*	*Alternaria*	*Rhizoctonia solani*	*Colletotrichum capsici*
NA1	ND	ND	ND	ND	ND	ND
NA2	ND	20.56	ND	ND	ND	25.98
NA3	11.53	ND	18.76	26.98	ND	18.98
NA4	ND	ND	ND	ND	ND	ND
NA5	ND	ND	ND	ND	ND	ND
NA6	ND	ND	ND	ND	ND	ND
NA8	ND	ND	ND	ND	ND	ND
BA1	21.42	13.35	11.45	ND	ND	21.65
BA2	14.81	ND	ND	19.76	16.76	15.87
BA3	ND	ND	ND	ND	ND	ND
BA5	ND	21.43	ND	ND	ND	ND
BA7	ND	ND	ND	ND	ND	ND
BA8	ND	ND	ND	ND	ND	ND
BA10	ND	ND	ND	ND	ND	ND
JA1	ND	ND	ND	ND	ND	ND
JA2	12.56	ND	ND	ND	ND	ND
JA3	ND	13.87	ND	ND	ND	28.97
JA5	ND	ND	ND	ND	ND	ND
JA7	30.76	27.43	25.67	35.71	37.07	30.62
JA9	14.81	ND	ND	19.76	16.76	15.87
DA1	ND	ND	ND	ND	ND	ND
DA3	ND	21.43	ND	ND	ND	ND
DA5	ND	ND	ND	ND	ND	ND
GA2	ND	ND	ND	ND	ND	ND
GA4	ND	ND	ND	ND	ND	ND
GA5	ND	ND	ND	ND	ND	ND
GA6	12.56	ND	ND	ND	ND	ND

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00004.7

The formation of a clear zone may be due to the secretion of an antifungal substance by the bacterial cell and may have spread to the medium indicating inhibition of fungal growth. Rhizobia not only play a major role in biological nitrogen fixation but also improve plant growth and reduce disease incidence in various crops. Rhizobia is known to control the growth of many soil-borne plant pathogenic fungi belonging to different species such as *Fusarium, Rhizoctonia, Sclerotium* and *Macrophomina*. The antagonistic activity of rhizobia is mainly attributed to the production of antibiotics, hydrocyanic acid (HCN), mycolytic enzymes and siderophores under iron-limiting conditions. Rhizobia are also reported to induce systemic resistance and increase the expression of genes related to plant defense, which effectively immunizes plants against pathogens (Das *et al.,* 2017). Haq and Ghaffar, (1993) also reported that *Rhizobium meliloti* did not affect *M. fazolina, R. solani* and *F. solani's* development stopped. *B. japonicum, M. phaseolina* and *R. solani* stopped. The results of these findings are also supported by Bardin *et al.* (2004). The strains R12 and R20, a PGPR dumping-off of Pythium causes the ability to induce resistance in field peas causing *Pythium* sp. Further studies are according to Hock *et al.* (2015) and Deepta *et al.* (2017) who pointed out that *R. leguminosarum* and *F. oxysporum* inhibits fungus.

CONCLUSION

The results concluded that the soil samples of Badli showed higher number of bacteria (297.21 $cfug^{-1}$ and the rhizobial isolate BA2 displayed maximum plant growth. The rhizobia also displayed significant variation in antifungal activities. Its isolate JA7 recorded maximum inhibition of 37.76 per cent for *R. Soloni* followed by 35.71 per cent for *Alternaria*.

ACKNOWLEDGMENT

The authors expressed their gratitude to National Mission on Himalayan Studies (NMHS) and MoEF & CC for financial assistance.

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Online Manuscript No. 97287 recived on 22.3.2022
Manuscript accepted for publication on 5.11.2022

Journal of Eco-friendly Agriculture **18**(1): 27-31. 2023
doi: 10.5958/2582-2683.2023.00005.9

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Growth, phenology and yield of Rapeseed (*Brassica rapa* L.) as affected by application of sulphur and organic manure

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ABSTRACT

Rapeseed (*Brassica rapa* L.) is one of the important annual oilseed crops grown worldwide for edible oil, animal fodder, vegetables, condiments and biodiesel. An experiment was conducted to study the effect of sulphur and organic manure on the growth, phenology and yield of Rapeseed at the Research Farm of Sant Baba Bhag Singh University, Jalandhar, India. The experiment consisted of three levels of sulphur (20, 40 and 60 kg/ha) and two levels of FYM (5 and 10 t/ha). The randomized block design was used with seven treatments and three replications. The higher number of seeds per siliqua, length of siliqua and seed yield were recorded in T_5 treatment (Sulphur 40 kg/ha and FYM 10 t/ha) as compared to the control. Test seed weight was higher in T_6 treatment (Sulphur 60 kg/ha and FYM 10 t/ha) as compared to the control. Overall, the results suggested that the improvement in growth, yield attributes and seed yield of rapeseed crop were observed in treatment T_5. However, the application of sulphur 60 kg/ha also showed improvement in the test weight of the rapeseed crop. The growth and yield attributes of rapeseed obtained from the treated plots were superior as compared to plots under control.

***Key words*:** Farmyard manure, Growth, Rapeseed, Sulphur, Yield.

Rapeseed-mustard is one of the important annual oilseed crops cultivated in India and grown worldwide (Rathore *et al.*, 2012). Rapeseed is an important source of edible oil, animal fodder, vegetables, condiments and biodiesel. Rapeseed plays a valuable role in securing edible oil production worldwide (Dong-Hui and Yan, 2016). India occupies the fourth position after Canada, China, and European Union in the area and production (Bhanu *et al.*, 2019). India produces almost 7.5 million tonnes of rapeseed mustard from the 6.5 million hectares area becoming the third largest producer in the world with a productivity of 1100 kg/ha (Kumar and Bala, 2013). The cultivated area under oilseeds in India is about 27 million ha out of which 72 per cent area is under rainfed conditions (Kumar *et al.*, 2018). It is grown as a cash crop in Haryana, Himachal Pradesh, Madhya Pradesh, Punjab, Uttaranchal and Western Uttar Pradesh (Kumar *et al.*, 2018).

Rapeseed is rich in minerals like calcium, manganese, copper, iron, zinc, vitamins A, B, C and proteins. The oil and protein of rapeseed-mustard seeds range from 40-48 and 20-40 per cent, respectively (Singh and Meena, 2017). Sulphur is the fourth essential secondary macronutrient after NPK for the growth, metabolism and development of all plants. Sulphur play important role in various physiological and biochemical functions in plants (Rathore *et al.*, 2012). Sulphur helps in the biosynthesis of oil, and the metabolism of carbohydrates, proteins and fats. Sulphur is also necessary for chlorophyll formation (Das *et al.*, 2016). The rapeseed-mustard crop has a higher requirement of sulphur. The increase in yield of rapeseed mustard by 12-48 per cent under irrigated conditions and by 17-24 per cent under rainfed conditions with the sulphur application. The maximum plant height, branches per plant, seeds per siliqua, test weight and yield of rapeseed were observed with the application of sulphur 60 kg/ha (Solanki *et al.*, 2016).

Farmyard manure (FYM) not only supplies macronutrients but meets the crop requirement of micronutrients and improves soil health (Sharma *et al.*, 2018). The FYM increase the growth and yield parameters of rapeseed mustard. Besides, help in increasing the availability of added inorganic nutrients (Kumar *et al.*, 2019). The application of FYM 5 t/ha increases plant height, the number of branches per plant and test weight of seed in rapeseed over control (Pathak and Pal, 2016). The combined application of sulphur and FYM plays an important role in the rapeseed-mustard crop. Several research workers' results showed that the application of sulphur and FYM enhances growth, yield and quality as well as soil health (Alam *et al.*,

2014). Therefore, the objective of the study was to assess the effect of sulphur and organic manure on the growth, phenology and yield of rapeseed crop.

MATERIALS AND METHODS

The experiment was carried out at Sant Baba Bhag Singh University Khiala, Jalandhar during the *Rabi* season of 2019-20. The farm is located at 31p 25' 37"N latitude 70p 84' 34"E longitude. The experiment was laid out in a randomized block design with seven treatments and three replications. The experiment contained three levels of sulphur (S_1=20 kg/ha, S_2=40 kg/ha and S_3=60 kg/ha) and two levels of Farmyard manure (F_1=5 t/ha and F_2=10 t/ha). The details of the treatment combinations were: T_1=Control, T_2=20 kg S/ha+5 t FYM/ha, T_3= 20 kg S/ha+10 t FYM/ha, T_4= 40 kg S/ha+5 t FYM/ha, T_5= 40 kg S/ha+10 t FYM/ha, T_6= 60 kg S/ha+10 t FYM/ha, and T_7= 60 kg S/ha+5 t FYM/ha.

The experimental field was ploughed well two times using the tractor and then levelled to ensure good germination. The field was cleaned properly and weeds were removed from the field for better performance of the field. The seed was sown at a spacing of 30 × 15 cm in plots of size 2.6 × 3.0 m with a seed rate of 5 kg/ha. A variety of rapeseed TL-15 was used for sowing. The calculated dose of sulphur and FYM for each plot was applied before the sowing. The sulphur was applied to the soil through Zinc Sulphate. The extra plants were thinned to ensure optimum plant population. The two weedings were done to remove the weeds from the field manually. The sowing was done during the last week of October and harvested in the last week of February (2020). Proper management practices were adopted to ensure good crop growth.

The various observations on growth attributes (plant height, leaves/plant and branches/plant), phenological stages (Days taken to 50% flowering, 100% flowering and days taken to maturity) and yield attributes (Number of seeds/siliqua, length of siliqua, test weight, biological yield, seed yield, stover yield and harvest index) were recorded from the randomly selected five plants during the crop period.

Statistical analysis

The data were statistically analyzed using software OPSTAT software developed by O.P. Sheoran, Computer Programmer at CCS HAU, Hisar India.

RESULTS AND DISCUSSION

Growth attributes

The various treatments had a significant effect on growth attributes such as plant height, number of leaves per plant and branches per plant (Table 1). The plant height had no significant effect on plant height at 30 and 60 days after sowing (DAS) except 90 DAS. The maximum plant height was observed (17.00 cm) in treatment T_3 at 30 DAS, (74.60 cm) in treatment T_5 at 60 DAS and (109.66 cm) in treatment T_5 at 90 DAS. The minimum plant height was observed in treatment under control. The plant height and other growth attributes increased in rapeseed crop (Table 1). Similar results were also documented by earlier workers (Solanki *et al*., 2016; Yadav *et al*., 2014; Alam *et al*., 2014; Piri *et al*., 2019). The various treatments had no significant influence on the number of leaves per plant at 30, 60 and 90 DAS. The maximum number of leaves per plant was observed in treatment T_5 at 30 DAS (7.00), 60 DAS (12.36) and 90 DAS (22.40). While the minimum number of leaves was observed in the control plot. The number of leaves and growth attributes increased in the rapeseed-mustard crop (Table 1). The results of the present investigation corroborate with Singh and Meena (2017) and Nath *et al.* (2018). The different treatments had no significant effect on primary branches/plant at 60 DAS.

Table 1. Effect of different treatments on growth attributes

Treatments	Plant height (cm)			Number of leaves/plant			Primary branches/plant		Secondary branches/ plant	
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	60 DAS	90 DAS	60 DAS	90 DAS
T_1	14.20	60.40	92.66	5.83	9.73	16.66	2.56	4.90	7.33	9.40
T_2	16.80	67.40	98.40	6.66	10.60	19.00	3.10	5.93	11.33	17.33
T_3	17.00	63.80	94.46	6.60	10.66	18.50	3.53	5.60	12.83	16.93
T_4	16.33	71.60	104.60	6.46	11.40	19.53	3.93	6.46	14.23	18.26
T_5	16.66	74.60	109.66	7.00	12.36	22.40	3.60	6.40	14.76	21.50
T_6	16.40	74.20	102.53	6.60	11.20	20.46	3.20	6.00	12.10	19.16
T_7	15.73	70.53	96.86	6.33	10.86	19.86	3.40	5.86	13.10	16.26
LSD (*P*=0.05)	NS	NS	8.36	NS	NS	NS	NS	0.91	2.13	2.63

T_1=Control, T_2=20 kg S/ha+5 t FYM/ha, T_3= 20 kg S/ha+10 t FYM/ha, T_4= 40 kg S/ha+5 t FYM/ha, T_5= 40 kg S/ha+10 t FYM/ha, T_6= 60 kg S/ha+10 t FYM/ha, and T_7= 60 kg S/ha+5 t FYM/ha; DAS: Days after sowing; NS: Non-significant; LSD: Least significant difference

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00005.9

The maximum number of primary branches per plant was recorded in treatment T_4 at 60 DAS (3.93) and 90 DAS (6.46) as compared to the control. The application of sulphur and FYM significantly enhanced the branches per plant in the rapeseed-mustard crop (Table 1). Similar observations were recorded by Kuotsu *et al.* (2014) and Parihar *et al.* (2010). The treatments had a significant influence on secondary branches per plant. The maximum number of secondary branches was observed in treatment T_5 at 60 and 90 DAS (14.76 and 21.50, respectively) (Table 1). Similar observations were recorded by Solanki *et al.* (2016) and Kuotsu *et al.* (2014).

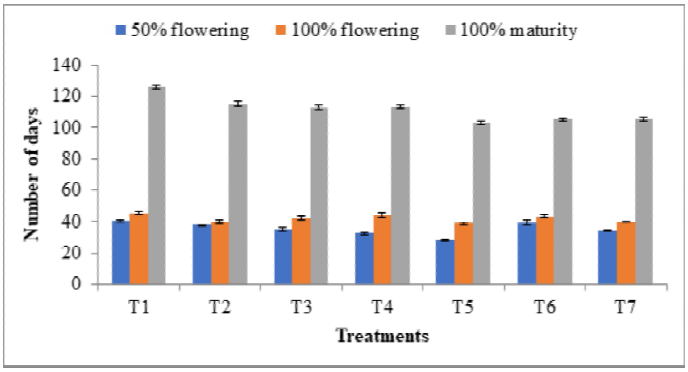
Phenological studies

The results related to the days taken to 50% flowering, 100% flowering and 100% maturity of rapeseed were influenced by various treatments (Fig. 1). The various treatments had no significant influence on the number of days taken to 50 and 100% flowering. The minimum days taken to 50 and 100% flowering were observed in treatment T_5 as compared to control and other treatments. Similar observations were recorded in the rapeseed-mustard crop (Nagdive *et al.*, 2007). The various treatments had a significant effect on days taken to 100% maturity. The treatment T_5 induced early maturity as compared to the control. These results are in agreement with the findings of Rehman *et al.* (2013), Muhammad *et al.* (2017) in the mustard crop.

Yield attributes

The data related to yield attributes were influenced by various treatments. The various treatments had a significant influence on the yield attributes such as seeds per siliqua, length of siliqua, siliqua per plant, test weight, seed yield, stover yield, harvest index and biological yield. The maximum number of seeds per siliqua (14.93) was recorded in treatment T_5 as compared to the control (Table 2). These results are in close conformity with the findings of Solanki *et al.* (2016) who reported an increased number of seeds per siliqua in the rapeseed-mustard crop. The higher length of siliqua (4.46 cm) was observed in treatment T_5 while the minimum was in the control plot (Table 2). Similar results were observed by Singh *et al.* (2007) in rapeseed crop. The maximum number of siliqua per plant (195.50) was observed in treatment T_4 as compared to the control (Table 2). These results are supported Solanki *et al.* (2016) and Kumawat *et al.* (2014).

The various treatments had a significant influence on the test weight of rapeseed. The higher test weight (3.53 g) was observed in treatment T_6 as compared to the control. Similar results were reported by Kapur *et al.* (2010). The maximum seed yield (16.46 kg/ha) was recorded in treatment T_5 while the minimum was observed in the control plot. The application of sulphur and FYM increased the seed yield of



[T_1=Control, T_2=20 kg S/ha+5 t FYM/ha, T_3= 20 kg S/ha+10 t FYM/ha, T_4= 40 kg S/ha+5 t FYM/ha, T_5= 40 kg S/ha+10 t FYM/ha, T_6= 60 kg S/ha+10 t FYM/ha, and T_7= 60 kg S/ha+5 t FYM/ha]

Fig. 1: Effect of different treatments on phenological studies in Rapeseed

Table 2. Effect of different treatments on various yield attributes

Treatments	Seeds/siliqa	Siliqua length (cm)	Siliqua /plant	Test weight (g)	Seed yield (q/ha)	Stover yield (q/ha)	Biological yield (q/ha)	Harvest index
T_1	9.53	2.93	165.33	2.43	8.76	20.16	30.06	18.93
T_2	13.00	3.20	180.33	3.13	11.66	39.40	34.60	20.93
T_3	10.86	3.53	185.50	3.36	12.26	42.50	35.60	21.06
T_4	14.66	3.86	195.50	3.40	14.36	41.33	35.36	22.30
T_5	14.93	4.46	191.83	3.30	16.46	46.93	37.03	23.66
T_6	14.20	3.76	188.43	3.53	15.53	43.76	36.36	22.26
T_7	14.13	3.80	186.10	3.43	14.53	41.26	34.83	22.46
LSD (P=0.05)	3.12	0.74	11.99	0.36	1.53	2.81	0.79	1.23

T_1=Control, T_2=20 kg S/ha+5 t FYM/ha, T_3= 20 kg S/ha+10 t FYM/ha, T_4= 40 kg S/ha+5 t FYM/ha, T_5= 40 kg S/ha+10 t FYM/ha, T_6= 60 kg S/ha+10 t FYM/ha, and T_7= 60 kg S/ha+5 t FYM/ha; LSD: Least significant difference

rapeseed (Alam *et al.,* 2014). Similar observations were also reported by Varenyiova *et al.* (2017) and Sattar *et al.* (2011) in the mustard crop. The higher stover yield (46.93 q/ha) was observed in treatment T_5 as compared to control and other treatments. These results corroborate the findings of Solanki *et al.* (2016) in rapeseed crop. The maximum biological yield (37.03 q/ha) was observed in treatment T_5 over control. These findings are in line with the results of Singh *et al.* (2016) and Singh *et al.* (2017) on the rapeseed-mustard crop. The maximum harvest index (23.66) was recorded in treatment T_5 while the minimum was observed in the control plot (Table 2). These findings are in agreement with the findings of Singh *et al.* (2016) and Singh *et al.* (2017) in the mustard crop.

CONCLUSION

In the present study application of sulphur and FYM on the rapeseed-mustard crop was evaluated. All the treated plots showed positive results as compared to the control. Initially, there was no difference in the growth parameters in the plots. However, during the vegetative stage, the treated plots showed a significant response as compared to the control. The various traits were significantly affected by the application of sulphur and FYM. The overall results suggested that the combined application of sulphur 40 kg/ha and FYM 10 t/ha resulted in an improvement in growth, yield attributes and seed yield in rapeseed crop. However, the growth and yield attributes of the rapeseed obtained from the treated plots were superior as compared to plots under control. It is concluded that the application of sulphur (40 kg/ha) along with FYM (10 t/ha) was the best treatment as compared to others. It is the most important agronomic practice for the successful growth, development and productivity of rapeseed.

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Online Manuscript No. 100635 recived on 23.8.2022
Manuscript accepted for publication on 19.11.2022

Journal of Eco-friendly Agriculture **18**(1): 32-36. 2023
doi: 10.5958/2582-2683.2023.00006.0

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Effect of levels of potassium on yield and economics of different blackgram varieties in Manipur sub-tropical condition

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ABSTRACT

The aim of this research was to determine the effect of different levels of potassium on the growth and yield of different blackgram varieties. A field experiment was conducted during *Kharif* 2021, at the College of Agriculture, Central Agricultural University, Imphal, Manipur. The soil of the experimental plot was clay in texture, nearly acidic in soil reaction (pH 5.96), medium organic carbon (0.75%), available N was low (280.39 kg/ha), medium available P_2O_5 (20.67 kg/ha) and available K_2O (188.16 kg/ha). There were 12 treatment combinations with two factors *viz*., Potassium levels (0, 20, 30, and 40 kg K_2O/ha) and Varieties (Pant U-31, Uttara, and CAU BG-1) laid out in a Factorial Randomized Block Design and were replicated thrice. The findings show that irrespective of varieties, grain yield (1125.67 kg/ha), stover yield (2410 kg/ha), harvest Index (34.74%), gross income (Rs 123823.70/ha), net income (Rs 83552.42/ha) and BC ratio (2.07) were maximum with the application of 40 kg K_2O/ha.

Key words **:** Blackgram, yield, economics, potassium, varieties

Blackgram (*Vigna mungo* L.) belongs to the family Leguminosae and is one of the most important pulse crops grown in Asian countries including India. Blackgram is native to India. Blackgram is called "*Urd*" in Hindi and locally known as "*Sagol hawai*" in Manipur. Blackgram fixes 30-40 kg/ha nitrogen in the soil. Blackgram contains about 24 per cent proteins, 60 per cent carbohydrates,10.9 per cent moisture,1.4 per cent fibre, 3.2 per cent minerals and vitamins *viz*., calcium- 154 mg, phosphorus- 385 mg, iron- 9.1 mg, and a small amount of vitamin B complex. Blackgram production has been distributed mainly in tropical to subtropical countries. It is grown in *kharif*, *rabi,* and summer seasons in India, Pakistan, Sri Lanka, and some countries of East Asia. In India, blackgram is very popular in Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, Uttar Pradesh, West Bengal, Punjab, Haryana, Tamil Nadu, and Karnataka. Various blackgram varieties are developed resistant to Yellow mosaic virus (YMV), powdery mildew, *Cercospora* leaf spot *etc*. IPU 94-1 (Uttara) is a short-duration variety developed by IIPR, Kanpur. It is a resistant variety to the yellow mosaic virus. Blackgram variety, Pant Urd-31 due to its durable resistance to YMV disease, photo-thermo insensitivity, short duration, dwarf type with balanced vegetative growth and higher yielding ability gained popularity among farmers across the country which is evident from the breeder seed indent received from different blackgram growing states of India (Singh *et al.,* 2015). To grow the crop successfully, a proper supply of nutrients is a very important and mainly essential macronutrient. Potassium is an essential macronutrient required for the proper development of plants. In addition to the activation of numerous enzymes, K plays an important role in the maintenance of electrical potential gradients across cell membranes and the generation of turgor. Potassium is one of the principal plant nutrients underlining crop yield production and quality determination. It is the most abundant cation in the plant, while involved in many physiological processes. Potassium's impact on water relations, photosynthesis, assimilate transport and enzyme activation can have direct consequences on crop production (Pettigrew, 2008).

MATERIALS AND METHODS

A field experiment was carried out during *Kharif* 2021, at the College of Agriculture, Central Agriculture University, Imphal, Manipur. The experimental site is situated at the College of Agriculture, C.A.U. Imphal campus. It is located at a latitude of 24°45′ N with a longitude of 93°54' E and an altitude of 774 meters above mean sea level. There were 12 treatment combinations with two factors *viz*., Potassium levels (K_0: 0, K_1: 20, K_2: 30, and K_3: 40 kg K_2O/ha) and Varieties (V_1: Pant U-31, V_2: Uttara, and V_3: CAU BG-1) laid out in a Factorial Randomized Block Design and were replicated thrice.

Treatment combinations were as follows:

T_1 = V_1 K_0 = Pant U-31 x K_2O @ 0 kg/ha
T_2 = V_1 K_1 = Pant U-31 x K_2O @ 20 kg/ha
T_3 = V_1 K_2 = Pant U-31 x K_2O @ 30 kg/ha
T_4 = V_1 K_3 = Pant U-31 x K_2O @ 40 kg/ha
T_5 = V_2 K_0 = Uttara x K_2O @ 0 kg/ha
T_6 = V_2 K_1 = Uttara x K_2O @ 20 kg/ha
T_7 = V_2 K_2 = Uttara x K_2O @ 30 kg/ha
T_8 = V_2 K_3 = Uttara x K_2O @ 40 kg/ha
T_9 = V_3 K_0 = CAU BG-1 x K_2O @ 0 kg/ha
T_{10} = V_3 K_1 = CAU BG-1 x K_2O @ 20 kg/ha
T_{11} = V_3 K_2 = CAU BG-1 x K_2O @ 30 kg/ha
T_{12} = V_3 K_3 = CAU BG-1 x K_2O @ 40 kg/ha

The observations *viz* seed yield (kg/ha), stover yield (kg/ha), harvest index (%), gross income (Rs/ha), net income (Rs/ha), and BC ratio were recorded with standard procedures. The data was statistically analyzed.

RESULTS AND DISCUSSION

Yield parameters

The results on yield parameters *viz*., seed yield (kg/ha), stover yield (kg/ha), and harvest index (%) are presented below.

Grain Yield (kg/ha)

Irrespective of varieties, with increased levels of potassium from zero to 40 kg K_2O/ha, there was a significant increase in the yield levels from 585.78 to 1059.22 kg/ha, respectively. Among the interactions, the plants that received 40 kg K_2O/ha recorded maximum grain yields of 1125.67, 1078.67, and 973.33 kg/ha in Pant U-31, Uttara, and CAU BG-1, respectively followed by 30 kg K_2O/ha. However, in absolute control plants, the lowest yield was recorded in Uttara (566 kg/ha) followed by Pant U-31(585 kg/ha) (Table 1). Potassium enhanced carbohydrate production and photosynthesis translocation, which contributes to the higher yield as reported by Chaudhari *et al*. (2018). High K levels activate the starch-synthesis enzyme, which then effectively transports starch from the sites of synthesis to the storage organs (Patil *et al*., 2011). The findings of the experiment are supported by Chettri and Mandai (2004), Kumar *et al.* (2004), and Hussain *et al*. (2011). Among varieties, significant results were recorded which was similar to the experiment performed on mungbean varieties by Burrio *et al*. (2015) and Hussian *et al*. (2011) in blackgram and also in trials at AICRP, MULLaRP on different blackgram varieties (Anonymous, 2020-21).

Stover Yield (kg/ha)

Irrespective of varieties, potassium levels *i.e.,* 20, 30, and 40 kg K_2O/ha recorded a stover yield of 2100.67, 2139.33, and 2173.00 kg/ha, respectively which were on par with each other. Among the interactions, significantly higher stover yield was recorded with the application of potassium @ 40 kg K_2O/ha in all three varieties *i.e.,* in Uttara (2410 kg/ha), Pant U-31 (2199 kg/ha), and CAU BG -1 (1910 kg/ha) which was at par with 30 kg K_2O/ha. The lowest stover yield was recorded in plants without fertilizer in CAU BG-1 (1500 kg/ha), Pant U-31 (1530 kg/ha), and Uttara (1744 kg/ha) (Table 2). Due to its involvement in tissue development, assimilation, transport, and storage, potassium plays a significant role in growth (Chakmak, 2010). Likewise, an increase in crop yield was brought on by the production of chlorophyll and photosynthesis, which in turn led to the accumulation of dry materials in stover yield as reported by Dadhich and Gupta (2004). Similar results were reported by Abraham *et al*. (2021) and Thakare (2016). Among varieties, a significant difference was recorded in stover yield. The findings are supported by Gangawar *et al*. (2012).

Harvest Index (%)

Irrespective of varieties, potassium levels *i.e.,* 20, 30, and 40 kg K_2O/ha recorded a harvest index of 31.87, 32.37, and 32.98 per cent, respectively which were on par with each other. There were noticeable differences between the genotypes. Among the interactions, the application of 40 kg

Table 1: Grain yield (kg/ha) as influenced by different potassium levels, different blackgram varieties and their interactions

Varieties Potassium levels	V_1 (Pant U-31)	V_2 (Uttara)	V_3 (CAU BG-1)	Mean
K_0 (0 kg K_2O/ha)	585.00	566.00	606.33	585.78
K_1 (20 kg K_2O/ha)	1032.67	997.67	600.67	977.00
K_2 (30 kg K_2O/ha)	1082.00	1035.33	945.67	1021.00
K_3 (40 kg K_2O/ha)	1125.67	1078.67	973.33	1059.22
Mean	956.33	919.42	856.50	-

Varieties		Pottassium		Interaction	
S.E(d).±	C.D. at 5%	S.E(d).±	C.D. at 5%	S.E(d).±	C.D. at 5%
5.57	11.56	7.43	15.41	22.29	46.22

Table 2: Stover yield (kg/ha) as influenced by different potassium levels, different blackgram varieties and their interactions

Varieties Potassium levels	V_1 (Pant U-31)	V_2 (Uttara)	V_3 (CAU BG-1)	Mean
K_0 (0 kg K_2O/ha)	1530.00	1744.00	1500.00	1591.33
K_1 (20 kg K_2O/ha)	2130.00	2342.00	1830.00	2100.67
K_2 (30 kg K_2O/ha)	2152.00	2386.00	1880.00	2139.33
K_3 (40 kg K_2O/ha)	2199.00	2410.00	1910.00	2173.00
Mean	2002.75	2220.50	1780.00	-
Varieties		Pottassium	Interaction	
S.E(d).±	C.D. at 5%	S.E(d).± C.D. at 5%	S.E(d).±	C.D. at 5%
72.06	149.45	83.21 172.57	144.12	298.91

Table 3: Harvest index (%) as influenced by different potassium levels, different blackgram varieties and their interactions

Varieties Potassium levels	V_1 (Pant U-31)	V_2 (Uttara)	V_3 (CAU BG-1)	Mean
K_0 (0 kg K_2O/ha)	27.00	24.50	29.05	26.85
K_1 (20 kg K_2O/ha)	33.03	29.58	32.99	31.87
K_2 (30 kg K_2O/ha)	33.40	30.25	33.47	32.37
K_3 (40 kg K_2O/ha)	33.85	30.34	34.74	32.98
Mean	31.82	28.67	32.56	-
Varieties		Pottassium	Interaction	
S.E(d).±	C.D. at 5%	S.E(d).± C.D. at 5%	S.E(d).±	C.D. at 5%
0.92	1.91	1.06 2.21	1.84	3.83

Table 4: Gross returns (Rs/ha) as influenced by different potassium levels, different blackgram varieties and their interactions

Varieties Potassium levels	V_1 (Pant U-31)	V_2 (Uttara)	V_3 (CAU BG-1)	Mean
K_0 (0 kg K_2O/ha)	64350.00	62260.00	66696.30	64435.43
K_1 (20 kg K_2O/ha)	113593.70	109743.70	99073.70	107470.40
K_2 (30 kg K_2O/ha)	119020.00	113886.30	104023.70	112310.00
K_3 (40 kg K_2O/ha)	123823.70	118653.70	107066.30	116514.60
Mean	105196.90	101135.90	94215.00	-

Table 5: Net returns (Rs/ha) as influenced by different potassium levels, different blackgram varieties and their interactions

Varieties Potassium levels	V_1 (Pant U-31)	V_2 (Uttara)	V_3 (CAU BG-1)	Mean
K_0 (0 kg K_2O/ha)	25818.72	23728.72	28165.02	25904.15
K_1 (20 kg K_2O/ha)	74242.50	70392.50	59722.50	68119.17
K_2 (30 kg K_2O/ha)	79258.72	74125.02	64262.42	72548.72
K_3 (40 kg K_2O/ha)	83552.42	78382.42	66795.02	76243.29
Mean	65718.09	61657.17	54736.24	-

Table 6: Benefit-cost ratio as influenced by different potassium levels, different blackgram varieties and their interactions

Varieties Potassium levels	V_1 (Pant U-31)	V_2 (Uttara)	V_3 (CAU BG-1)	Mean
K_0 (0 kg K_2O/ha)	0.67	0.62	0.73	0.67
K_1 (20 kg K_2O/ha)	1.88	1.78	1.51	1.72
K_2 (30 kg K_2O/ha)	1.99	1.86	1.62	1.82
K_3 (40 kg K_2O/ha)	2.07	1.95	1.66	1.89
Mean	1.65	1.55	1.38	-

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00006.0

K_2O/ha recorded the highest harvest index in all three varieties *i.e.,* CAU BG-1 (34.74 g), Pant U-31 (33.85 g), and Uttara (30.34 g) followed by plants that received 30 kg K_2O/ha and 20 kg K_2O/ha which were on par with each other. Significantly lowest values of harvest index were recorded in all three varieties *viz*., Uttara (24.50%), Pant U-31 (27.00%), and CAU BG-1 (29.58%) which received no fertilizers (Table 3). An increase in harvest index signals that dry matter is being partitioned more evenly between the reproductive regions of the plant, increasing plant production (Palaniappa, 1985).

Economics

The results on economics of treatments *viz.,* gross income (Rs/ha), net income (Rs/ha), and benefit cost ratio are presented below.

Gross Income (Rs/ha)

Gross return was observed higher with the application of 40 kg K_2O/ha in Pant U-31 variety (Rs 123823.70/ha) followed by 20 kg K_2O/ha in Pant U-31 variety (Rs 119020.00/ha). In the absolute control plot, Uttara recorded lower gross returns (Rs 62260.00/ha). In all three varieties under trial, Pant U-31 with different levels of K recorded the highest gross income (Table 4). A similar result was shown by Asghar *et al*. (1984) in blackgram.

Net Income (Rs/ha)

A similar trend as that of gross return was followed in net returns also where higher net returns were observed in the application of K at 40 kg/ha in Pant U-31 (Rs 83552.42/ha) followed by the application of K at 30 kg/ha in Pant U-31 (Rs 79258.72/ha). A lower net return was recorded without the application of fertilizer in Uttara (Rs 23728.72/ha) (Table 5).

Benefit-cost Ratio

Maximum BC ratio was recorded in the application of K at 40 kg/ha in Pant U-31 variety (2.07) followed by the application of K at 30 kg/ha in Pant U-31 (1.99). A lower BC ratio was observed in absolute control in Uttara (0.62) followed by Pant U-31 (0.67). This showed the application of potassium at 40 kg/ha improved the plant growth and yield but also improved the benefit-cost ratio in all three varieties in comparison to lower levels. Whereas the lowest net return (Rs 23728.72/ha) and BC ratio (0.62) were recorded in control with Uttara variety (Table 6). A similar result was recorded by Patil and Dhonde (2009) in greengram, Salve and Gunjal (2011) in groundnut, and Thesiya *et al*. (2013) in blackgram for the cost of cultivation, gross income, net income, and BC ratio.

CONCLUSION

From the experimental results, it can be concluded that the application of potassium significantly influenced on yield and economics of different blackgram varieties. The application of 40 kg K_2O/ha improved growth and yield significantly in all three varieties. A maximum increase in yield with the application of potassium was recorded in Pant U-31 followed by Uttara and CAU BG-1. Maximum gross income, net income, and BC ratio were recorded with the application of 40 kg K_2O/ha in Pant U-31, followed by Uttara and CAU BG-1.

ACKNOWLEDGEMENT

The first author wishes to express gratitude to the Chairperson of the Advisory Committee Dr. Jamkhogin Lhungdim and all faculty members of the Department of Agronomy, College of Agriculture, Central Agriculture University, Imphal, Manipur for their excellent guidance, boundless encouragement, and providing all necessary facilities required for the experiment.

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Online Manuscript No. 100505 recived on 11.8.2022
Manuscript accepted for publication on 19.11.2022

Journal of Eco-friendly Agriculture **18**(1): 37-42. 2023
doi: 10.5958/2582-2683.2023.00007.2

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Green synthesis and characterization of zinc oxide nanoparticles

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ABSTRACT

Green synthesis of zinc oxide nanoparticles (ZnO-NPs) is a novel and non-toxic process that replaces harmful existing physical and chemical methods. The synthesized nanoparticles with C.*reticulata* peel powder were characterized with visual analysis, UV-visible spectroscopy and PSA for nanoparticle confirmation. The maximum UV-visible spectral absorption peaks were observed in aqueous extract 357 nm compare to ethanol extract 340.2 nm wavelengths. Aqueous extract showed greater particle size with 55 nm, whereas the particle size in ethanol extract was found to with 75.8 nm with ZnO-1 precursor. Similarly, an average particle size of 76.7 nm in aqueous extract was observed in ZnO-2 precursor, whereas in ethanol extract it is 82.2 nm. As a result of the experimental study, water has been identified as an universal, safe, and environmentally friendly solvent with an optimal and acceptable solvent system for green synthesis of zinc oxide nanoparticles.

Keywords: Green synthesis, *Citrus reticulate,* Zinc oxide nanoparticles (ZnO NPs), Extract, Precursor

Nanotechnology is at the forefront of modern research and it will eventually allow us to create custom-made materials and goods with new enhanced features, new nanoelectronics components, new sorts of "smart" medications and sensors, and even electronic-biological interfaces. Nanofabrication uses two methods to carve smaller materials out of bigger ones. One is a top-down approach to miniaturizing current technologies, while the other is a bottom-up approach in which a massive nanostructure is created at the atomic level using various chemical, physical, or biological interactions (Das *et al*., 2017).

Metal nanoparticles synthesis is a fascinating subject in nanoscience. Several research groups have been interested in various forms of metal NPs, including as iron oxide, silver nitrate, copper oxide and zinc oxide, in the previous few decades. Metal oxides such as TiO2, ZnO, MgO, and CaO are of particular importance among inorganic materials since they are not only stable under extreme conditions, but are also usually recognized as safe materials for humans and animals. Among this zinc oxide (ZnO) nanomaterials have remarkable potential for applications in solar cells, sensors, piezoelectric devices, photodiode devices, sunscreens, and anti-reflection coatings and photocatalysis (Sharma *et al.,* 2020). It also exhibits effects by inducing ROS generation and causing apoptosis. ZnO NPs shows synergistic and enhanced therapeutic efficacy when combined with other therapeutic agents. ZnO nanoparticles have also been explored as drug carriers for anti-cancer drugs. Zinc oxide is a modifier in the textile industry because of its use for the production of safety garments and all kinds of fabric for technical applications (Mishra *et al*., 2017).

Metal nanoparticles are synthesized using different approaches, including the sol-gel method, thermal decomposition, hydrothermal and microwave irradiation and many more. However, due to the formation of a large number of secondary waste products as a result of the incorporation of chemical agents for the reduction process, these chemical and physical synthesis procedures are time-consuming, costly, and toxic (Gupta *et al.,* 2018). Metal nanoparticles synthesized from plants and/or plant extracts are more stable than those produced by other organisms. Modified organisms have a strong ability to optimize the synthesis of additional proteins, enzymes, and biomolecules that are required for nanoparticle production and stability. Thus, *Citrus reticulata,* the mandarin organge peel was selected as a catalyst for synthesis of green zinc oxide nanoparticles. Citrus fruits are commonly used in food processing industries, where tons of pulp peel and rags are thrown solid waste during the extraction process. The fruit waste generation not only results in financial losses but also adds to the expense of waste management and disposal (Bisht *et al.,* 2020). Thus to minimize the fruit wastage and protect the environment, an experimental study was conducted by using *citrus reticulata* peel as a catalyst in the green synthesis of zinc oxide nanoparticles different solvent extracts.

MATERIALS AND METHODS

Orange fruits of the Nagpur variety were collected from the local market of Dharwad. Collected fruits were cleaned

thoroughly using distilled water, peeled and their edible portions were seperated. The peels were oven-dried at 35°C for 48 h, and ground to a fine powder with a laboratory grinder, sieved with ASTM standard mesh of 600 microns of 8 number size and stored in an air container for further experimentation (Mahmoud *et al.,* 2016). Approximately 43.4 g pod powder was obtained from one kg of orange fruit.

For aqueous extraction, 100ml of Milli Q water was taken in the beaker and heated until the temperature rises to 100°C with a magnetic stirrer and then 2 grams of *Citrus reticulata* peel powder was added to the beaker and kept for stirring at 100 °C for 20 minutes. After cooling to room temperature, the extract was filtered through whatman paper no.1.

Two grams of weighed powder was also extracted with 25ml of solvent, incubated under agitation at 25 °C for 24hrs. Centrifuged at 5000 rpm at 5 °C for 10 min and refrigerated. The supernatant obtained was separated and the residue was re-extracted with a fresh 25 ml of solvent. The process was repeated and the supernatants were pooled and the extract obtained was measured and filtered using whatman filter paper no. 1. Both the extracts were stored in refrigeration for further analysis.

Zinc acetate dihydrate (ZnO-1) and zinc nitrate hexahydrate (ZnO-2) were the two precursors selected for study with *Citrus reticulata* powder as catalyst for the green synthesis of zinc oxide nanoparticles. For the green synthesis of zinc oxide nanoparticles, zinc acetate dihydrate and zinc nitrate hexahydrate precursors were dissolved in Milli-Q water and stirred for four to five hours by adding *C. reticulata* peel extract. Sodium hydroxide was added drop by drop to maintain the pH of the solution (Chitha *et al.,* 2015).

The characterization of green synthesized zinc oxide nanoparticles (ZnO NPs) was carried out for different parameters such as visual analysis, UV-Visible spectrophotometer and particle size analyzer. The optimized treatment concentrations obtained during the study were taken for further characterization with the following instruments.

Absorbance peak of synthesized and standard ZnO NPs was measured using UV-Visible spectrophotometer. It works on the principle of Beer-Lambert law. This law states that whenever a beam of monochromatic light is passed through a solution with an absorbing substance, the decreasing rate of the radiation intensity along with the thickness of the absorbing solution is proportional to the concentration of the solution and the incident radiation and it is expressed in the equation

$$A = \log (I_o/I) = ECL$$

Where,

A = Absorbance

I_o = Intensity of light upon sample cell (cd)

I = Intensity of light departing from sample cell (cd)

E = Molar absorptivity (L/mol. m)

C = Concentration of solute (mol/L)

L = Length of sample cell (m)

From the Beer-Lambert law, it was determined that the greater the number of the molecules that were capable of absorbing light at a certain wavelength, the greater the extent of the absorption of light was observed. An optical spectrophotometer records the wave lengths at which absorption occurs, together with the degree of absorption at each wavelength. The wave length that corresponds to the highest absorption is usually referred to as λ_{max}. The resulting spectrum was presented as a graph of absorbance (A) versus wavelength (λ). For ZnO NPs the absorbance peak (λ_{max}) ranges from 300 to 400 nm (Santhoshkumar *et al.* 2017).

The sample was prepared by diluting 1 mL of ZnO NPs into 2 mL of distilled water and measuring the UV-Visible spectrum of solutions (UV probe Version 2.61) (Habibi *et al.*, 2017). The settings used in UV-Visible spectrophotometer are given in following Table.

Particle size of synthesized ZnO NPs was measured using PSA (Plate 3). The zetasizer (nano series) performs size measurements using a process called Dynamic Light Scattering (DLS). The principle of DLS is that fine particles and molecules are in constant random thermal motion, called Brownian motion, which diffuse at a speed related to their size, smaller particles diffuse faster than larger particles. The speed of Brownian motion is also determined by the temperature, therefore precision temperature control is essential for accurate size measurement. The measurement of the particle can be observed from 0.6 nm to 8.9 µm. The synthesized ZnO nanoparticle samples were subjected to sonication to obtain a homogenous population of ZnO

Settings for absorbance peak in UV-Visible spectrophotometer

Measurement parameter	Value
Wavelength range (nm)	300 to 400
Scan speed	Medium
Sampling interval	0.20
Auto sampling interval	Enabled
Scan mode	Auto

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00007.2

nanoparticles. Then samples were analyzed through Particle Size Analyzer (Nicomp, NANOZ Z3000 PSS) to determine mean size and per cent distribution. (Ahmed *et al*., 2016).

RESULTS AND DISCUSSION

Visual analysis of aqueous and ethanol extracts

The study revealed a colour change with aqueous and ethanol extracts. For the preliminary confirmation, the change in color of the ZnO NPs was observed from pale yellow to dark yellowish mud brown in ZnO-1 ZnO NPs extracted with aqueous extract, whereas with ethanol extract it was from pale yellow to yellowish brown. The visual analysis with ZnO-2 with aqueous extract showed the change of the colour from pale yellow to dark yellowish green and for ethanol extract it is orangish brown, depicted in Plates 1 to 4.

Adding *C. reticulata* peel powder to zinc acetate dihydrate and zinc nitrate hexahydrate leads to physio-chemical changes in the aqueous solution. The most apparent of them is a change in the colour of the reaction mixture after 2 hours, which is regarded as an early marker for the formation of NPs. The transformation may be due to the formation Zn ions to ZnO NPs is hypothesized to be mediated by flavonoids and phenolic substances (Manokari *et al*., 2019). The colour of the solution stopped changing after a few hours, indicating that the ZnO salt had been completely bio-reduced into ZnO nano particles. It was also observed that the change in colour was high in aqueous with dark yellowish mud brown than in ethanol extract with pale yellow to yellowish brown.

UV–Visible spectroscopic analysis

UV spectroscopy or UV–visible spectrophotometry (UV–Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum. Both extracts were subjected to this examination. It is discovered that using ZnO-1 precursor, aqueous extraction produced a greater peak at 90 °C with 357 nm, similarly for ethanol extract a peak is appeared at 330 nm, as shown in Fig. (1) and (2).

The peak at 340.2 nm is absorbed in ZnO-2 for ethanol extraction. A peak at 352nm was detected in aqueous extract with zinc nitrate. On comparing the absorption peaks of water and ethanol, it was clear that the absorption peak for water was significantly more tailing than ethanol extract as depicted in Fig. (3) and (4). The results were in line with (Khoza *et al*., 2012) who reported that the antioxidant

Plate 1 Colour change of ZnO NPs with ZnO-1 precursor (aqueous extract)

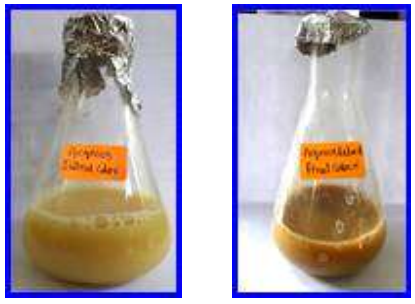


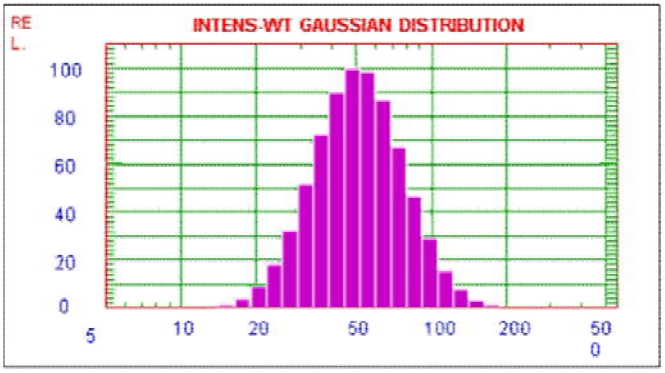
Plate 3 Colour change of ZnO NPs with ZnO-2 precursor (aqueous extract)

Plate 2 Colour change of ZnO NPs with ZnO-1 precursor (ethanol extract)

Plate 4 Colour change of ZnO NPs with ZnO-2 precursor (ethanol extract)

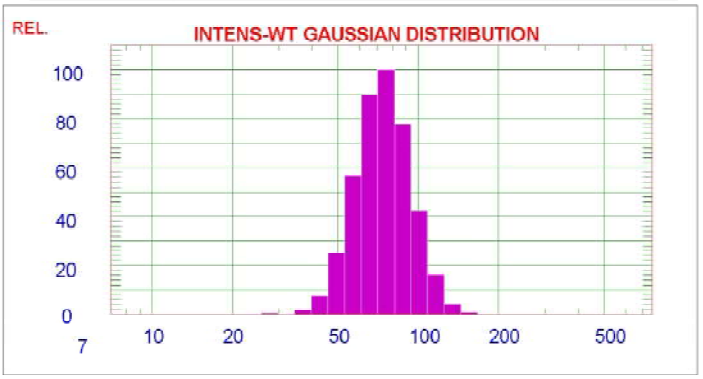
Intensity-Weighted gaussian distribution analysis

Gaussian summary:			
Mean Diam	= 55.0 nm	Variance (P.I.)	= 0.177
Stnd.D	= 23.2 nm (42.1%)	Chi Squared	= 25.218



Particle size distribution aqueous extract

Gaussian summary:			
Mean Diam	= 75.8 nm	Variance (P.I.)	= 0.058
Stnd. D	= 18.3 nm (24.1%)	Chi Squared	= 10.457

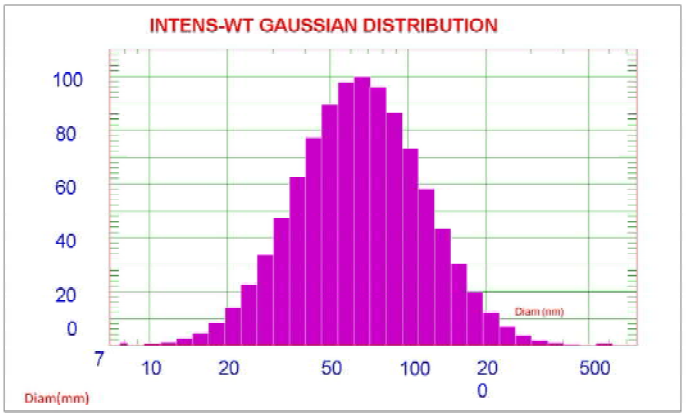


Particle size distribution ethanol extract

Fig. 5: Particle size distribution of zinc oxide nanoparticles with ZnO-1 precursor

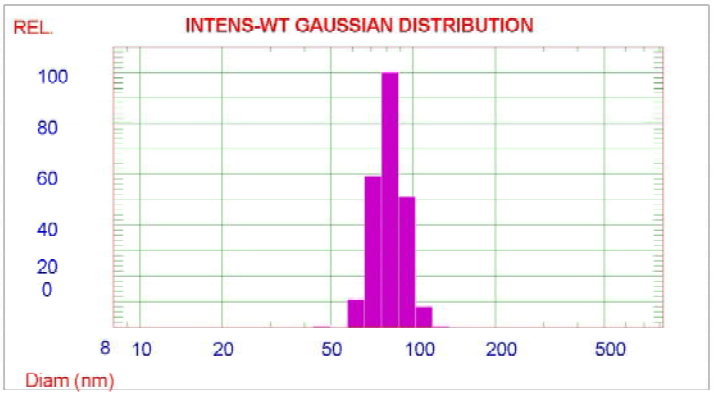
Intensity-Weighted gaussian distribution analysis

Gaussian summary:			
MeanDiam	= 76.7 nm	Vari (P.I.)	= 0.327
Stnd. D	= 43.9 nm (57.2%)	Chi Squared	= 75.392



Particle size distribution with aqueous extract

Gaussian summary:			
Mean Diam	= 82.2 nm	Vari (P.I.)	= 0.017
Stnd. D	= 10.9 nm (13.2%)	Chi Squared	= 128.630



Particle size distribution ethanol extract

Fig. 6: Particle size distribution of zinc oxide nanoparticles with ZnO-2 precursor

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00007.2

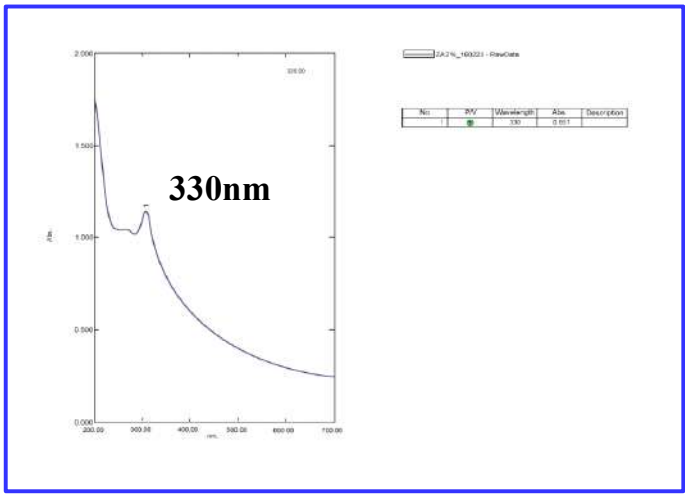


Fig. 1: Absorption peak of ethanol extract with ZnO-1 precursor at 2 per cent

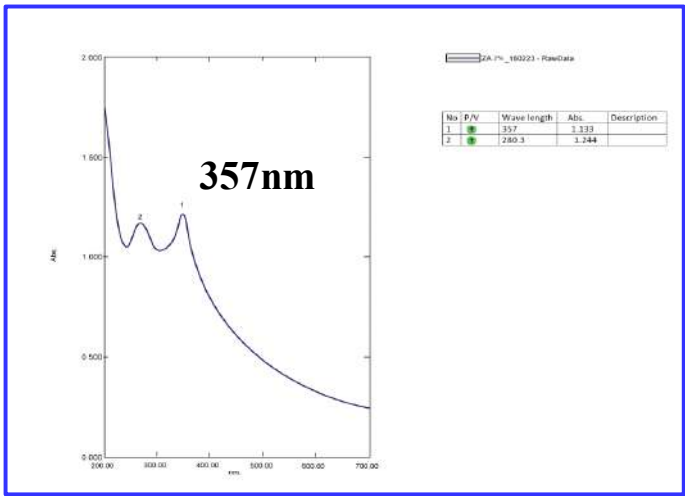


Fig. 2: Absorption peak of aqueous extract with ZnO-1 precursor at 2 per cent

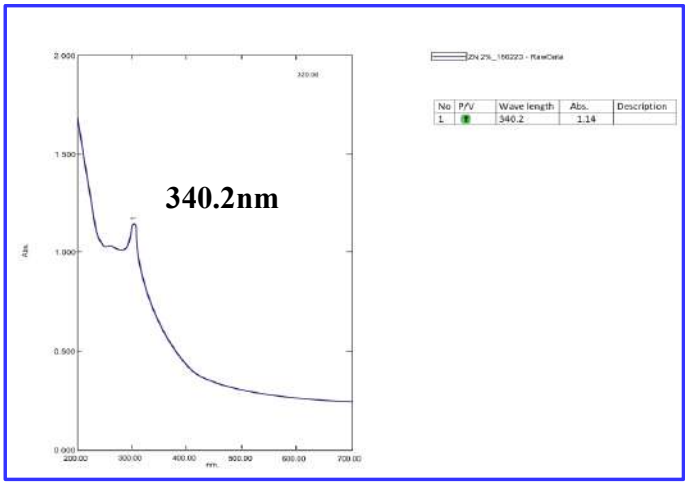


Fig. 3: Absorption peak of ethanol extract with ZnO-2 precursor at 2 per cent

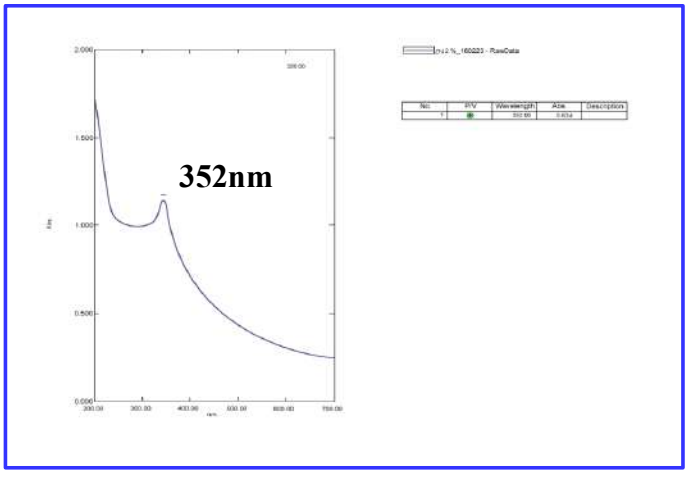


Fig. 4: Absorption peak of aqueous extract with ZnO-2 precursor at 2 per cent

capacities of plant extract greatly depends on extract composition as well as conditions and mechanism of the test used. Water as a solvent forms highly crystalline ZnO nanoparticles which are star shaped. The polar characteristic of the solvent was proposed to be the main factor that affects both nucleation and growth of ZnO nanoparticles and, consequently, determines the shape, size and aspect ratio of the products. Further, it is noted that the absorption peak for water is also much more tailing compared to the peaks of ethanol. Tables 1 and 2 show the results of the experiments.

The UV-Vis spectrum of green synthesized ZnO NPs characterized the influence of surface plasmon resonance due to the significant changes in the absorbance maxima which indicates the interaction of phytochemicals with zinc ions within the plant extract and change the color of the reaction (Mohan *et al*., 2016). The absorption peak for Zinc oxide is prominent in the wavelength range (330 and 357 nm) due to band-gap absorption, which occurs when electrons are removed from the valence band and transferred to the conduction band. In addition, as ZnO particles are nano-scale and the particle size distribution is limited, a strong absorption peak appears at (200-400) nm.

Bhramarambica and Abhinav (2019) obtained similar results by producing ZnO nanoparticles from an aqueous extract of *Rhizophora mucronata* leaves and testing their antibacterial activity against a variety of microorganisms. The synthesized ZnO nanoparticles were analyzed using UV/VIS spectroscopy, and a peak at 350 nm confirmed the purity of the ZnO NPs.

Particle size analyzer (PSA)

Particle size analysis is used to characterize the size distribution of particles in a given sample. The particle size of ZnO-1 precursor in aqueous extract was found to be low with an average particle size of 55 nm, whereas the particle size in ethanol extract was found to be high at 75.8nm, according to the PSA results from Fig. (5). Similarly, an average particle size of 76.7 nm in aqueous extract was observed in ZnO-2 precursor whereas in ethanol extract it is 82.2 nm. Fig. (6) shows intensity-weighted Gaussian distribution synthesized ZnO nanoparticles.

According to the results, nanoparticles produced at 2 percent concentration of catalyst with ZnO-1 precursor showed reduction in particle size when compare to ZnO-2 precursor. The reduction in particle size may be due several factors, such as pH of the reaction mixture, reaction time, stirring speed, nature of capping agents, and concentration of metal precursors, which greatly affect the properties of the zinc oxide nanoparticles and their applications (Shaba *et al.,* 2020). In green nano synthesis, water plays a crucial role during evolution of crystal structure precisely at nucleation and growth phases by encompassing via sub-phases, namely, elemental distribution within metal particles, self-assembly, particle aggregation and coalescence and formation of pores in nanomaterial's, respectively (Ali *et al.,* 2020).

CONCLUSION

In the present study, the green ZnO-NPs were synthesized using *Citrus reticulate* peel powder with aqueous and ethanol extracts. As a preliminary confirmation, visual observance of the reaction mixture was obtained. The color change was stronger in aqueous with dark yellowish mud brown than in ethanol extract with pale yellow to yellowish-brown, which influenced the absorption peak and size of the nanoparticles. The size of the nanoparticle also differed with extraction. PSA results revealed that in both the precursors, water extract has a lower particle size than ethanol extract. Water was identified as an universal, safe, and environmentally friendly solvent with an optimal and acceptable solvent system for green synthesis of zinc oxide nanoparticles. Based on the results of experimental study, the aqueous extract method was chosen for optimization of green zinc oxide nanoparticles for further study.

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Online Manuscript No. 99265 recived on 27.8.2022

Manuscript accepted for publication on 16.11.2022

Journal of Eco-friendly Agriculture **18**(1): 43-50. 2023
doi: 10.5958/2582-2683.2023.00008.4

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Influence of priming and irrigation intervals on growth and fruit yield of okra [*Abelmoschus esculentus* (L.) Moench]

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ABSTRACT

The field experiment conducted at the experimental farm, Faculty of Agricultural Sciences, DAV University, Jalandhar in spring-summer of 2019 in split plot design with 3 replications and 12 treatment combinations (comprising of four priming treatments *viz*., dry seed, hydro-priming, 3% KCL and 3% KNO_3 and three irrigation intervals *viz*., 5, 8 and 11 days intervals) showed that the irrigation given at 5 days intervals proved best with respect to minimum number of days to 50 per cent emergence, number of days to 50 per cent flowering, days to first picking and recorded maximum for inter-nodal length, number of nodes, plant height, number of pickings, number of fruits per plant, average fruit weight, fruit yield, root length and chlorophyll "b" while, irrigation at 8 days intervals recorded maximum number of branches per plant. Among priming treatment, hydro-priming recorded maximum inter-nodal length, number of fruits per plant, average fruit weight and fruit yield while, minimum days to 50 per cent flowering, number of picking were observed in 3 per cent KCL and 3 per cent KNO_3 recorded minimum days to first picking of fruits and maximum inter-nodal length, number of branches per plant, maximum plant height, maximum number of pickings, longest root length and chlorophyll "b". Among treatment combinations, 5 days intervals × 3 per cent KNO_3 recorded minimum number of days to 50 per cent emergence, maximum number of branches per plant, while 5 days intervals × 3 per cent KCL recorded minimum number of days to first picking, number of fruits per plant, average weight of fruit and highest yield which was at par with 5 days intervals × hydro-priming and 5 days intervals × dry seed. All the irrigation intervals performed best during shorter irrigation intervals as compared to longer irrigation intervals. It was found from the results of this research that hydro-priming and halo-priming enhanced okra growth and yield parameters. It was concluded that problems of low germination and production in okra can be improved by hydro-priming and halo-priming.

Keywords: Okra, Seed priming, Dry seed, Hydro-priming, Halo-priming, Irrigation intervals.

Okra (*Abelmoschus esculentus* L. Moench), one of the most widely grown vegetable crop of the family Malvaceae (Naveed *et al*., 2009), is cultivated over an area of 5.33 lakh hectare, with annual production of 63.46 lakh million tones in India (Anonymous, 2019a). In Punjab, it is cultivated over an area 5.30 thousand hectare with annual production 55.39 thousand tonnes (Anonymous, 2020b). It is also grown in Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Haryana and Himachal Pradesh. It contains Vitamin A and C and is also rich source of iron and calcium (Pandita *et al*., 2010) and iodine (Pal *et al*., 1952). Okra green pods are consumed as salads, vegetables, soups and stews, fresh or dried, fried or boiled (Ndunguru *et al*., 2004). Okra seeds are source of oil and protein and have been used in small scale for oil production (Oyelade *et al*., 2003). In some countries, okra is used in folk medicine as antiulcerogenic, gastro-protective and diuretic agent (Gurbuz *et al*., 2003). In the plains of north India, it is mainly grown during rainy season (June-July) and spring summer (February-March). The main problem in early spring summer planting is delayed and erratic seed germination of seed (Pandita *et al*., 2010) which is attributed to its hard seediness. The percentage of hard seediness increases with maturity and decrease with moisture content (El-Balla *et al*., 2011).

Seed priming is a pre-sowing seed treatment in which the seed is soaked in water in order to start pre-germination processes (Posmyk *et al*., 2001). The process increases seed germination (Kausar *et al*., 2009) and thereby improve seedling stand enabling better crop establishment (Job *et al*., 2000). It is a simple, low cost and effective approach for early seedling growth and yield under stressed and non-stressed conditions. It triggers the synthesis or activation of some enzymes that catalyze the mobilization of storage reserves in seed and also allows some of the metabolic processes to occur before the actual germination get started (Farooq *et al*., 2008). Hydro-priming and osmo-priming promote the proliferative and dynamic growth of roots (Carceller and Soriano, 1972).

The specifications for irrigation intervals and the amounts of water needed for the plants must be managed in order to increase productivity and reduce mineral soil washing and avoid water stress (Boras *et al*., 2011). It permits the dissolution of the mineral element into various parts of

the plant (El-Sahookie *et al*., 2009). Apart from germination, another problem that occurs during spring-summer season is requirement of adequate moisture in the soil. Irrigations at 5 to 6 days interval are given to summer crop to help continue fruiting. But exposing the crop for a certain level of water stress either during a specific period or throughout the growing season helps in maximizing water-use efficiency. On the other hand, saved water can be used to irrigate other crops (Bahadur *et al*., 2005). Okra farmers in Punjab are facing major problem of seed germination and decrease in water resources during peak summers. Keeping these points in view, the effect of priming, irrigation intervals and their combination on growth and yield of okra was studied.

MATERIALS AND METHODS

The present study was carried out at the experimental farm of Faculty of Agricultural Sciences, DAV University, Jalandhar in 2019 to study the "Effect of priming and irrigation intervals on growth & fruit yield of Okra [*Abelmoschus esculentus* (L.) Moench]". The soil of experimental site was alkaline in reaction (pH 8.02), sandy loam in texture and available N, P and K were 207.80, 16.73, 54.56 kg ha^{-1}, respectively. The field experiment was conducted in split plot design with factorial concept with three replications. The treatments comprised of four priming treatments (dry seed, hydro-priming, 3 per cent KCL and 3 per cent KNO_3) and three irrigation intervals (5 days intervals, 8 days intervals and 11 days intervals) constituting 12 combinations viz., I_1P_1 (5 days interval × dry seed), I_1P_2 (5 days interval × hydro-priming), I_1P_3 (5 days interval × 3% KCL), I_1P_4 (5 days interval × 3% KNO_3), I_2P_1 (8 days interval × dry seed), I_2P_2 (8 days interval × hydro-priming), I_2P_3 (8 days interval × 3% KCL), I_2P_4 (8 days interval × 3% KNO_3), I_3P_1 (11 days interval × dry seed), I_3P_2 (11 days interval × Hydro-priming), I_3P_3 (11 days interval × 3% KCL), I_3P_4 (11 days interval × 3% KNO_3). The entire recommended dose of phosphorus, potassium and FYM and one-third nitrogen was applied at the time of field preparation while the remaining nitrogen was applied in two equal split doses at 30 and 45 days of sowing. All the cultural and management practices were followed whenever needed. Five random plants were selected per plot for the collection of data. Observations were recorded on growth (days to 50% emergence, days to 50 per cent flowering, number of nodes per plant, inter-nodal length, plant height, number of branches per plant, root length, chlorophyll a and b) and yield attributes (days to first picking, total number of pickings, number of fruits per plant, average fruit weight, fruit yield per plant, fruit yield per plot and fruit yield per hectare). The data recorded during the course of investigation was subjected to statistical analysis using factorial split plot design (Panse and Sukhatme, 1985). The interpretation of result was based on F test and critical difference (CD) at 5 per cent level of significance.

RESULTS AND DISCUSSION

Days to 50 per cent emergence

The days to 50 per cent emergence recorded under different levels of irrigation indicated that the minimum number of days to emergence was observed under I_1 (8.11 days) which were significantly lower than I_3 (10.78 days). However, no significant difference was observed between I_1 and I_2 (Table 1). The present findings are in corroboration with Gunawardhana and Silva, (2011) who reported reduction in seedling emergence rate due to water stress. Ayeni *et al*. (2015) also reported that germination of okra seeds occurred best in cultivated soils when they were irrigated at four days interval. The effect of priming treatments on days to 50% emergence was found non-significant. The days to 50 per cent emergence were minimum with I_1P_4 (7.00 days) which was significantly lower than I_1P_1 (9.33 days) but was found to be at par with I_1P_2 and I_1P_3. This might be due to the fact that rapid imbibitions of primed seeds resulting in increased seed metabolism thus increasing the germination rate. It is also evident that the days to 50 per cent emergence were found to be at par under all priming treatments at I_2 and I_3 level of irrigation. However, at I_3 level of irrigation the days for 50 per cent emergence were significantly higher with P_4 (13.44 days). The beneficial effects of priming may be more evident under unfavorable rather than favorable conditions (Parera and Cantliffe, 1994; Bradford, 1995) but due to occurrence of rain 5 days after sowing the okra plants did not experience water stress during germination and seedling emergence in the field.

Days to 50 per cent flowering

The minimum number of days to 50 per cent flowering was recorded in I_1 (50.08 days) which was significantly superior over the remaining treatments. The maximum number of days was recorded with I_3 (55.83 days). All the treatments were significantly different from each other. It might be due to sufficient availability of moisture which enabled them to utilize resources efficiently (Table 1). Similar results were also reported by (Aliyu *et al*. 2016) who observed that the shorter irrigation intervals gave plants chance to grow and attain flowering stage within fewer days. Similar findings were also reported by (Gudugi *et al*. 2012). Among the priming treatments, the minimum number of days was observed in P_3 (50.89 days). The treatment P_2 and P_4 were found to be at par with P_3. The treatment P_1 took maximum number of days to 50 per cent flowering (54.89 days). Similar findings were also reported by (Kaur *et al*. 2015; and Ullah *et*

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doi: 10.5958/2582-2683.2023.00008.4

al. 2002 b) who noted that primed crops emerged faster, flowered earlier and gave higher yield.

Number of nodes plant^{-1}

The data pertaining to number of nodes per plant as influenced by different levels of irrigation and priming revealed irrigation intervals significantly influenced the number of nodes per plant. The maximum number of nodes per plant was obtained with I_1 (17.83) which were significantly superior to I_2 and I_3. The next best treatment was I_2 which was also significantly better than I_3. (Table 1). These findings are in line with the findings of (Tan *et al* 2009) who also reported that amount of irrigation has significant effect on number of nodes per plant in bottle gourd.

However, the priming treatments and interactions I × P and P × I were found non-significant.

Inter-nodal length (cm)

The perusal of data (Table 1) showed that inter-nodal length was significantly influenced by irrigation intervals and priming treatments in okra. The maximum inter-nodal length was obtained with I_1 (7.79 cm) which was significantly higher to I_2 (6.31 cm) and I_3 (5.19 cm). However, I_2 and I_3 both were found significantly at par with each other. Among the priming treatments, maximum inter-nodal length was observed in P_2 and P_4 (6.80 cm) followed by P_3 (6.66 cm). All the priming treatments were significantly higher over P_1 (5.45 cm). The interactions among irrigation intervals and priming treatments were found to be non-significant.

Plant height (cm)

The tallest plants (68.68 cm) were obtained with I_1 which were significantly taller than plants obtained from I_2 and I_3 (Table 1). The shortest plants (58.29 cm) were obtained with I_3. Therefore, it is evident from the data that plant height decreased with increase in irrigation intervals. This reduction might probably have been due to increase in moisture stress condition with increase irrigation interval which in turn reduced the rate of cell enlargement and plant growth. El-Kader *et al*. (2010) also observed that height of okra increases by augmentation of water availability. They reported decrease in morphological characters like plant

Table 1. Effect of irrigation intervals on growth parameters in different priming treatments of okra

Irrigation intervals	Priming treatments	Days to 50 per cent emergence	Days to 50 per cent flowering	Number of nodes	Internodal length (cm)	Plant height (cm)	Number of branches plant^{-1}	Root length (cm)	Chlorophyll "a"	Chlorophyll "a"
5 days interval	Dry seed	9.33	51.67	17.93	6.27	65.83	1.40	7.70	4.12	2.44
	Hydro-priming	8.44	50.33	16.43	8.55	69.57	1.40	7.86	6.40	1.90
	KCL (3%)	7.67	47.33	20.43	7.70	70.43	1.47	8.46	5.77	1.62
	KNO_3 (3%)	7.00	51.00	16.53	8.64	68.87	2.33	8.58	6.83	2.40
Mean		8.11	50.08	17.83	7.79	68.68	1.65	8.15	5.78	2.09
8 days interval	Dry seed	8.00	55.00	17.97	5.51	54.37	1.47	9.10	6.02	1.54
	Hydro-priming	9.00	52.00	15.60	6.61	63.33	2.07	9.73	5.12	1.48
	KCL (3%)	9.44	50.33	14.67	6.61	58.97	1.60	10.61	6.34	1.36
	KNO_3 (3%)	8.67	50.00	15.00	6.52	68.83	2.27	10.11	5.01	1.36
Mean		8.78	51.83	15.81	6.31	61.38	1.85	9.88	5.62	1.44
11 days interval	Dry seed	10.33	58.00	13.53	4.57	51.80	1.20	10.30	4.44	1.95
	Hydro-priming	9.11	55.67	13.33	5.25	62.97	1.23	11.20	4.94	1.87
	KCL (3%)	10.22	55.00	11.07	5.67	58.83	1.33	10.75	4.31	1.58
	KNO_3 (3%)	13.44	54.67	14.37	5.25	59.87	1.30	11.96	5.06	2.23
Mean		10.78	55.83	13.08	5.19	58.29	1.27	11.05	4.69	1.91
Irrigation intervals										
SE m±		0.27	0.34	0.64	0.42	1.71	0.07	0.28	0.32	0.11
CD 0.05		1.09	1.09	1.82	1.19	4.88	0.22	0.81	NS	0.32
Priming treatments										
SE m±		0.52	0.66	0.97	0.51	2.68	0.10	0.29	0.40	0.17
CD 0.05		NS	1.39	NS	1.09	5.66	0.20	0.62	NS	0.36
Priming × Irrigation										
SE m±		0.61	1.14	1.68	0.89	4.63	0.16	0.51	0.69	0.29
CD 0.05		1.96	NS	NS	NS	NS	0.37	NS	1.59	NS
Irrigation × Priming										
SE m±		0.54	1.04	1.59	0.87	4.36	0.16	0.53	0.68	0.28
CD 0.05		2.03	NS	NS	NS	NS	0.37	NS	1.55	NS

height by increasing the irrigation interval. A similar observation was reported by (Al-Ubaydi *et al*. 2017 and Ghannad *et al.* 2014). Among the priming treatments, P_2, P_3 and P_4 were found to be significantly superior to P_1 (dry seed). The tallest plants were obtained with P_4 (65.76 cm) which was followed by P_2 (65.29 cm) and P_3 (62.74 cm) while shortest plants were obtained with P_1 (57.33 cm). Shah *et al*. (2011) also reported that seed priming of okra increased its plant height. However, these conclusions are not in agreement with Hegazi *et al.* (2014) who reported that osmo-priming is more efficient than hydro-priming. Osmo-priming causes significant increase in plant height in okra (Omran *et al*. 1980). The interactions of irrigation intervals and priming treatment on plant height were found to be non-significant.

Number of branches plant^{-1}

The number of branches per plant (Table 1) were maximum under I_2 (1.85) which were at par with I_1 (1.65). The minimum number of branches were (1.27) recorded with I_3 and both I_2 and I_1 were significantly superior to I_3. This may be due to prolonged water stress experienced by the plants with 11 days interval, because moisture is necessary for the physiological development, translocation and nutrient uptake of okra. El-Kader *et al*. (2010) also reported that branches per okra plant decreased when subjected to stress conditions, and highest number of branches per plant were obtained at the shortest irrigation interval. Similar findings were reported by (Asadipour and Madani 2014; and Aliyu *et al*. 2016) who also observed that with the higher number of branches produced the plants with short intervals may be as result of benefits derived from sufficient provision of moisture which enable them to utilize resources efficiently. Among the priming treatments, the maximum number of branches (1.97) was observed in P_4 which was significantly superior over other treatments. However, the treatment P_3 (1.47) was found to be at par with P_1 (1.36). Similar results were also reported by (Hegazi *et al*. 2014). Among the interactions of irrigation intervals and priming treatments, the number of branches were significantly higher in I_1P_4 (2.33) followed by I_2P_4 (2.27) and I_2P_2 (2.07) which were statistically at par among themselves. Irrespective of the priming treatments, the numbers of branches were lower with increased irrigation intervals and the differences were also significant except P_1.

Days to first picking of fruit

As evident from Table 2 the days to first picking of fruit were significantly influenced by irrigation intervals, priming treatments and their interactions I×P and P×I. Among the irrigation intervals, all the treatments were found to be significantly different from each other. The minimum (53.25 days) and maximum (58.83 days) number of days to first picking of fruits were observed in I_1 and I_3, respectively. With respect to priming treatments, minimum days for first picking were recorded in treatment P_4 (53.78 days). It was also revealed that P_4 was significantly superior to P_2 and P_1 but found to be at par with P_3. The maximum days for first picking of fruits were observed with P_1 (57.89 days). Among the interactions, I_1P_3 was significantly superior to I_1P_1 but was found to be at par with I_1P_2 and I_1P_4. At the same level of I_3, the treatment I_3P_4 was found to be significantly superior to all the remaining treatments.

Number of pickings

The irrigation intervals significantly influenced number of pickings in okra (Table 2). The maximum number of picking were observed in I_1 (13.33) followed by I_2 (12.50) and I_3 (12.00). I_1 was found to be significantly superior to I_2 and I_3 while, the remaining treatments were found to be at par with each other. Among the priming treatment, the maximum number of picking were observed in P_3 (13.11) followed by P_2 and P_4 (13.00) while minimum number of pickings were obtained in P_1 (11.33). It is apparent from the Table 4.8 that neither of the interaction, P × I or I × P significantly influenced the total number of pickings in okra.

Number of fruits plant^{-1}

The number of fruits per plant decreased with increase in irrigation intervals. Maximum number of fruits per plant (9.24) was observed in I_1 which was significantly higher than I_2 and I_3 (Table 2). All the treatments were significantly different from each other. This might be due to the prolonged moisture stress experienced by plants irrigated at 11 days interval hence resulting in less productivity. Water stress before and at the beginning of the flowering stage reduces the number of pods. In addition to water stress, high temperature and low humidity level also reduce the number of fruits per plant (Al-Harbi *et al.* 2008). The results are in agreement with Radder, (2008) and Anant, (2009) and Khan *et al*. (2005). Among priming treatments, maximum number of fruits per plant were observed in P_2 (8.84) followed by P_4 and P_3. All these treatments were significantly superior to P_1 (dry seed), which produced minimum number of fruits per plants (8.01). Similar results were also reported by Tania *et al*. (2020). Primed seeds with hot water had generally encouraged smooth germination over non-primed and other treatments due to which more yield was obtained from plants raised from hot water primed seeds. Mohammadi *et al*. (2009) had also reported that priming caused an increase in yield. The difference between primed and unprimed seeds in case of number of fruits per plant was two or five which may not be important to small farmers but obviously be a big

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doi: 10.5958/2582-2683.2023.00008.4

difference for commercial farmers. Several studies reported that seed priming enhance plant vigor and subsequently its yield. Thus, primed okra seeds improved yield (Rahman *et al*. 2016). Taken together these results, it is suggested that hydro-priming and halo-priming can improves the yield of okra. P × I or I × P interaction effects had significant influence on number of fruits per plant. I_1P_3 produced maximum number of fruits per plant (10.07) which were at par with I_1P_2 (9.38) and I_1P_4 (9.16). I_3P_1 produced maximum number of fruits per plant (7.40).

Average fruit weight (g)

The average fruit weight (Table 2) was significantly influenced by irrigation intervals, priming treatments and their interactions. Among irrigation intervals, maximum average fruit weight was observed in I_1 (9.88 g) which was significantly superior to I_2 and I_3. The higher pod weight recorded from 5 and 8 days irrigation intervals could be as a result of availability of moisture for photosynthesis and translocation of photosynthates for pod development which is limited at 11 days interval. West (2004) also reported that frequent irrigation increase size and weight of fruit. Among priming treatments, P_2, P_4 and P_3 registered significantly higher average fruit weight of 9.43 g, 9.39 g and 8.97 g, respectively. All treatments were significantly superior over P_1 (8.38 g). Interactions also had a significant impact on the average fruit weight. The highest average fruit weight was obtained in I_1P_3 (11.09 g) which was significantly superior over remaining treatments. The next best treatment was I_1P_2 (10.27 g). In case of osmo-priming the average fruit weight was not affected by the irrigation intervals but in hydro-priming and dry seeding the differences is significantly different.

Fruit yield plant^{-1} (g), plot^{-1} (kg) and hectare^{-1} (q)

The fruit yield per plant as well as per plot and per hectare (Table 2) was significantly influenced by different irrigation intervals. I_1 gave significantly highest fruit yield (71.06 g) plant^{-1}, (14.21 kg plot^{-1}), (157.92 q ha^{-1}) followed by I_2 (63.55 g plant^{-1}), (12.71 kg plot^{-1}), (141.22 q ha^{-1}) and I_3 (58.52 g plant^{-1}), (11.70 kg plot^{-1}), (130.04 q ha^{-1}). On the other hand, I_2 was found to be at par with I_3. The significant reduction of yield with increase in irrigation interval may also be due to overall effects of water on okra development at vegetative and reproductive phases. Total yield of okra per hectare significantly decreased with increase in irrigation interval, this indicated negative effect of water stress which reduced okra total yield per hectare by accelerating leaf senescence. Thus, drought stress has been reported to reduce translocation of assimilate from the leaves to the fruits. Reduction in total yield with increasing irrigation interval may also be due to corresponding effect of irrigation interval on yield component such as number of fruits per plant, number of branches, plant height which all responded in the same manner. Bahadur *et al*. (2009) and Alieyu *et al*. (2016) also reported reduction in okra yield with increased water stress. Among priming treatments, the maximum fruit yield was obtained with P_2 (68.99 g plant^{-1}), (13.80 kg plot^{-1}), (153.31 q ha^{-1}) followed by P_3 (67.86 g plant^{-1}), (13.57 kg plot^{-1}), (150.79 q ha^{-1}) and P_4 (67.49 g plant^{-1}), (13.50 kg plot^{-1}), (149.99 q ha^{-1}). All the priming treatments were significantly higher than dry seeding (control). Priming influenced seed germination and plant growth which contributed directly or indirectly in better yield performance in field. Jisha *et al*. (2013) also reported that priming improved uniform seed germination and seedling establishment in various crops. Rapid and synchronous field emergence of seedlings are two important pre-requisites to increase yield (Finch-Savage, 1995). Beneficial effects of hydro-priming on seedling emergence, biological yield and grain yield per unit area have also been reported by Kahlon *et al*. (1992) in wheat, Bastia *et al*. (1999) and Hussain *et al*. (2006) in sunflower. The interactions between irrigation intervals and priming treatments had significant effect on fruit yield per plant. The higher fruit yield per plant was produced by I_1P_3 (78.47 g plant^{-1}), (15.70 kg plot^{-1}), (174.37 q ha^{-1}) which was significantly superior over the remaining treatments. I_1P_2 (71.75 g plant^{-1}), (14.35 kg plot^{-1}), (159.44 q ha^{-1}) and I_1P_4 (70.35 g plant^{-1}), (14.07 kg plot^{-1}), (156.33 q ha^{-1}) were found to be at par with each other but all treatments were significantly higher than I_1P_1 (63.69 g plant^{-1}), (12.74 kg plot^{-1}), (141.54 q ha^{-1}). At I_2 irrigation interval, all priming treatments were significantly superior to dry seeding (control) which gave lowest fruit yield. Similar trend was also observed with I_3 irrigation interval and priming treatments.

Root length

The irrigation intervals and priming treatments significantly influenced the root length (Table 1). The lowest root length was observed in I_1 (8.15 cm) followed by I_2 (9.88 cm). The longest root length was observed in I_3 (11.05 cm). Similar results were also reported by Hussein *et al*. (2011). Among the priming treatments, the lowest root length was observed with P_1 (9.03 cm) followed by P_2 (9.60 cm) and P_3 (9.94 cm). The longest root length was observed with P_4 (10.22 cm). The osmo-priming and hydro- priming have been reported to promote vigorous root growth and shoot growth in soybean and cumin (Yuan-Yuan *et al*. 2010). However, the effect of priming and interaction between irrigation intervals and priming treatments were found to be non-significant.

Chlorophyll "a"

Chlorophyll "a" was influenced non-significantly by irrigation intervals and priming treatments (Table 2). However, the interactions were significant. The maximum content of chlorophyll "a" were observed in I_1P_4 (6.83 mg) which was at par with I_1P_2 (6.40 mg), I_2P_3 (6.34 mg) and I_2P_1 (6.02 mg) but significantly higher than remaining treatments. Similar results were also reported by Hegazi *et al*. (2014).

Chlorophyll "b"

The effect of irrigation intervals and priming treatments on chlorophyll "b" content was found to be significant. From the Table 2, it is revealed that the maximum chlorophyll "b" content was recorded in I_1 (2.09 mg) followed by I_3 (1.91 mg) and I_2 (1.44 mg). Among the priming treatments, maximum content of chlorophyll "b" was recorded with 3% KNO_3 (2.06 mg) followed by dry seed (1.97 mg) and hydro-priming (1.75 mg). The minimum content of chlorophyll "b" was observed in 3 per cent KCL (1.52 mg). Similar results were also reported by Hegazi *et al*. (2014). The priming enhancement effect of photosynthetic pigments has been suggested to be attributed to an improved germination performance and plant tolerance under temperature or water stress that would be reflected as enhanced growth (Chen and Arora 2011). This could be further reinforced where stomata conductance and relative chlorophyll contents of melon plants resulting from primed seeds were almost higher than those of the corresponding unprimed ones (Sivritepe *et al.* 2005). However, the interactions P × I or I × P were found to be non-significant for Chlorophyll "b".

CONCLUSION

It was concluded that the irrigation given at 5 days intervals proved best with respect to minimum number of days to 50 per cent emergence, number of days to 50 per cent flowering, days to first picking of fruits and recorded maximum inter-nodal length, number of nodes, plant height, number of pickings, number of fruits per plant, average fruit weight and fruit yield, longest root length and chlorophyll "b" while irrigation at 8 days intervals recorded maximum number of branches per plant. Among priming treatments, hydro-priming recorded maximum inter-nodal length, number of fruits per plant, average fruit weight and fruit yield while minimum days to 50 per cent flowering, number of picking were observed in 3 per cent KCL. The 3 per cent KNO_3 recorded minimum days to first picking of fruits and

Table 2. Effect of irrigation intervals on fruit yield and yield parameters in different priming treatments of okra

Irrigation intervals	Priming treatments	Days to first picking	Number of pickings	Number of fruits plant^{-1}	Average fruit weight (g)	Fruit yield kg plant^{-1}	Fruit yield kg plot^{-1}	Fruit yield q ha^{-1}
5 days interval	Dry seed	54.67	11.67	8.35	8.02	63.69	12.74	141.54
	Hydro-priming	53.67	14.00	9.38	10.27	71.75	14.35	159.44
	KCL (3%)	51.33	14.33	10.07	11.09	78.47	15.70	174.37
	KNO_3 (3%)	53.33	13.33	9.16	10.16	70.35	14.07	156.33
Mean	53.25	13.33	9.24	9.88	71.06	14.21	157.92	
8 days interval	Dry seed	57.00	11.33	8.30	9.01	50.62	10.13	112.50
	Hydro-priming	55.67	12.67	8.98	9.69	70.83	14.17	157.40
	KCL (3%)	55.67	13.00	8.20	8.69	64.59	12.92	143.52
	KNO_3 (3%)	54.00	13.00	8.12	8.28	68.17	13.63	151.49
Mean	55.58	12.50	8.40	8.91	63.55	12.71	141.22	
11 days interval	Dry seed	62.00	11.00	7.40	8.12	45.22	9.05	100.48
	Hydro-priming	60.33	12.33	8.16	8.35	64.39	12.88	143.10
	KCL (3%)	59.00	12.00	8.18	8.41	60.51	12.10	134.47
	KNO_3 (3%)	54.00	12.67	8.45	8.48	63.96	12.80	142.14
Mean	58.83	12.00	8.04	8.34	58.52	11.70	130.04	
Irrigation intervals								
SE m±		0.48	0.22	0.17	0.32	1.25	0.25	2.77
CD 0.05		1.36	0.61	0.49	0.92	3.55	0.71	7.90
Priming treatments								
SE m±		0.83	0.56	0.22	0.36	1.58	0.32	3.50
CD 0.05		1.77	1.19	0.48	0.76	3.34	0.67	7.41
Priming × Irrigation								
SE m±		1.44	0.97	0.39	0.62	2.73	0.55	6.07
CD 0.05		3.19	NS	0.89	1.44	6.22	1.24	13.82
Irrigation × Priming								
SE m±		1.34	0.87	0.38	0.63	2.67	0.54	5.94
CD 0.05		2.96	NS	0.86	1.46	6.09	1.22	13.53

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00008.4

maximum inter-nodal length, number of branches per plant, maximum plant height, maximum number of pickings, longest root length and chlorophyll "b". Among treatment combinations, 5 days intervals × 3 per cent KNO_3 recorded minimum number of days to 50 per cent emergence, maximum number of branches per plant, while 5 days intervals × 3 per cent KCL recorded minimum number of days to first picking of fruits, number of fruits per plant, average weight of fruit and highest yield which was at par with 5 days intervals × hydro-priming and 5 days intervals × dry seed. All the irrigation intervals performed best during shorter irrigation intervals as compared to longer irrigation intervals. It was found from the results of this research that hydro-priming and halo-priming enhanced okra growth and yield parameters. It can also be concluded that slow germination problems and the maximization of okra production can be improved by hydro- priming and halo-priming.

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Online Manuscript No. 97001 recived on 4.3.2022

Manuscript accepted for publication on 11.11.2022

Journal of Eco-friendly Agriculture **18**(1): 51-54. 2023
doi: 10.5958/2582-2683.2023.00009.6

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Estimation of resource use efficiency of chickpea production in Banda district of Bundelkhand region, Uttar Pradesh

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ABSTRACT

The Bundelkhand region of Uttar Pradesh is the major chickpea growing region of India were it is the main livelihood of the farmers dependent on agriculture in general. The Bundelkhand region comprises of 13 districts of which 7 are in Uttar Pradesh and 6 in Madhya Pradesh. The major pulse crops grown in the region are pigeon pea, urdbean, and mungbean cultivated during *kharif* and the chickpea, field pea and lentil in the *rabi* season. The study was carried out in of Baberu block in district Banda having largest area under chickpea. The coefficient of determination revealed that independent variables i.e. man-days x_1, seed x_2, and PPM x_5 estimated were significant at the 1 per cent level of significance and the sum of the elasticity's indicated the diminishing returns to scale. While the MVP of different inputs was found underutilized of chickpea production in the study area.

Keywords: *Resource Use Efficiency, Coefficients of Determination, MVP and Diminishing Return to Scale, etc.*

Chickpea, grown for centuries is the most important pulse crop of India in terms of area and for nearly 40 per cent of the total pulse production in the country. Its use as food in developing countries accounts for about 90 percent of global human pulse consumption. The pulses contribute about 10 percent of the daily protein and almost 5 per cent of energy intake in the peoples diet of One hundred grams of mature boiled chickpea contains 164 calories, 2.6 grams' fat of which only 0.27 grams is saturated, 7.6 grams of dietary fiber, and 8.9 grams of protein and complex carbohydrates. The grain legumes are produced by over 75 percent of rural farming households mainly for subsistence and little surplus is sold to generate cash income (Nassary *et al.* 2019). According to the Indian Institute of Pulse Research, vision document, India's population by 2030 is expected to touch 1.68 billion and the total pulse requirement by then would be of 32 million tonnes with the average productivity about 781 kg ha^{-1}, much below the world productivity of 871 kg ha^{-1}, to meet its domestic requirement (R.V. Chavan *et al.* 2020).

The UP Bundelkhand region comprises seven districts of southern UP i.e. Jhansi, Lalitpur, Jalaun, Hamirpur, Mahoba, Banda, and Chitrakoot, where agriculture is the most predominant occupation in the region and availability of land used for cultivation in the region is considered of lower fertility than in other agriculture zones of Uttar Pradesh. In Western part of Uttar Pradesh, for instance, over 75 percent of the total area is used for cultivation. Such high coverage is seen only in the Bundelkhand plain sub-region, in Hamirpur, Jalaun, and Banda districts; over 70 percent of the total area is used for cultivation in Jhansi and Mahoba districts. However, due to the large area of wasteland in the Bundelkhand intermediate region and Bundelkhand upland sub-regions, the percentage of land used for cultivation falls drastically to around 50 percent in Chitrakoot and Lalitpur districts.

This region as one of the potential producers of pulses and oilseeds is considered the bowl of pulses in Uttar Pradesh and is also notified for the tremendous production and productivity of pulses and oilseeds in general and chickpea in particular in the country (Hasan *et al.* 2018 and Kumar *et al.* 2018). The region also has drawbacks of having poor quality of soil with inadequate availability of inputs, responsible for low productivity. However, the chickpea crop has the capability of its being cultivated even at low inputs. They enter symbioas with nitrogen fixing bacteria and use fixed atmospheric nitrogen to improve the physical and biological properties of the soil.

MATERIALS AND METHODS

The estimation of resource use efficiency among chickpea growers in the Banda District of Bundelkhand Region (U.P.) was done adoping purposive sampling procedure. Selection of the district, Blocks and villages were done randomly. Each village having 20 chickpea growers

were selected randomly and a total sample size of 120 was selected for data collection. The Chickpea growers were interviewed using a pre-tested schedule for the collection of primary data. The data of chickpea growers collected during the *rabi* season in the agriculture year 2019-20 were compiled and the resource use efficiency of chickpea production was analyzed by Cobb-Douglas Production Function (CDPF).

The Cobb–Douglas production functionis, also known as a non-linear production function, was used for estimating the resource use efficiency in chickpea production by the chickpea growers. The function not only showed the resource use efficiency but also helped to readjust the selected variable for getting profit. To study resource use efficiency among chickpea growers, a modified Cobb - Douglas production function was fitted in dependent and independent variables for chickpea production studied using the formula detailed below:

$$Y = a\, x_1^{b1}.x_2^{b2}.x_3^{b3}.x_4^{b4}.x_5^{b5}.eo\ldots\ldots\ldots\ldots x_n^{bn}$$

Where,

Y = Gross value of output (main product + by-product) estimated at market price inrupees/quintal.

a = Constant term

Independent Variable

X_1 = Value of Human labour in (MD)

X_2 = Value of Seed in (Kg)

X_3= Value of Fertilizers in (Rs.)

X_4 = Value of Irrigation in (Rs.)

X_5= Value of Plant protection measures in (Rs.)

e = Error/ disturbance term

B_1 to B_5 = elasticity coefficients of respective inputs or regression coefficients of factor input.

The Cobb-Douglas type of production function was converted into log-linear form and the coefficients were estimated by using the ordinary least squares (OLS) method. In logarithmic form, it assumed a log-linear equation is as follows:

$$\text{Log } Y = \log a + b_1 \log x_1 + b_2 \log x_2 + b_3 \log x_3 + b_4 \log x_4 + b_5 \log x_5 + b \log \ldots\ldots\ldots$$

From the Cobb-Douglas production functions, the MVP of each resource was estimated and the marginal productivity of the particular input "X_1" at geometric mean of input and output was expressed in the following equation.

$$\text{MVP Xi} = \text{bi}\ \frac{\bar{Y}}{Xi} py\ (price\ of\ output)$$

Where,

Y = Geometric mean of output (Y).

Xi = Geometric mean of i^{th} input

bi = Regression coefficient i^{th} input

Py = Price of output

RESULTS AND DISCUSSION

Chickpea growers with their limited available resources desires to achieve maximum farm returns. The Cobb-Douglas production function or double log production function was fitted to find out the resources use efficiency with different inputs used (man-days x_1, seed x_2, fertilizer application x_3, irrigation x_4, and plant protection measure x_5) equally to the economic opportunities and make rational use of resources for the optimum level of the yield of chickpea in Banda district of Bundelkhand region Uttar Pradesh.

The estimation of regression coefficients of the chickpea (table 1) indicates that the value of the coefficient of determination (R^2) varies between 0 to 1, the highest (0.86) estimated in small size of the group which means that the 86 percent of the dependent variable explained by the independent variable (Asmatoddin *et al*. 2009 and Sengar *et al.* 2018). The seed x_2 was found significant at a 1 percent level of significance with the 0.05 standard error in a small

Table 1: Estimation of regression coefficients of Chickpea production

Items	Size of groups				Overall n = 120
	Marginal n = 32	Small n = 27	Medium n = 36	Large n = 25	
R^2	0.75	0.86	0.74	0.78	0.77
Intercept	0.64	0.33	-0.22	-1.80	-0.29
Variables					
Man - days (X_1)	0.16**	0.02	-0.04	0.12	0.11**
	(0.05)	(0.05)	(0.05)	(0.11)	(0.03)
Seed (X_2)	0.33**	0.48**	0.57**	0.73**	0.45**
	(0.05)	(0.05)	(0.07)	(0.15)	(0.04)
Fertilizer application (X_3)	0.02	0.01	-0.0002	-0.01	0.003
	(0.03)	(0.01)	(0.02)	(0.04)	(0.01)
Irrigation (X_4)	-0.03	-0.03	0.04*	0.03	0.02
	(0.06)	(0.02)	(0.02)	(0.04)	(0.01)
PPM (X_5)	0.002	0.04	0.03	0.12	0.07**
	(0.03)	(0.02)	(0.02)	(0.05)	(0.01)
∑ bi	0.49	0.48	0.61	0.73	0.63

**Significant 1 percent level, *Significant 5 per cent level Figures in parenthesis show standard error.

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00009.6

size group. However, the elasticity of coefficients indicates the increase in 1 percent seed rate will increase the yield by 0.48 percent and the sum of the elasticity indicates diminishing returns to scale. The coefficients of determination R^2, the least 0.74 in medium size of the group, means that it contributes 74 percent dependent variable explained by independent variables which are estimated significant. The seed x_2 and irrigation x_4 were found significant with standard error 0.07 and 0.02, respectively. Hence, the elasticity coefficients indicate that the 1 percent increase in seed x_2 will increase the yield by 0.57 percent, and the 1 percent increase irrigation x_4 will increase 0.04 percent in the yield, respectively.

The sum of the elasticity indicates diminishing returns to scale and the overall size of groups coefficient of determination R^2 was found 0.77 which means 77 percent dependent variable explained by an independent variable which is estimated significant man-days x_1, seed x_2, and plant protection measure (PPM) x_5 at 1 percent level of significance with standard error 0.03, 0.04 and 0.01, respectively. The elasticity of the coefficients indicates that the 1 percent

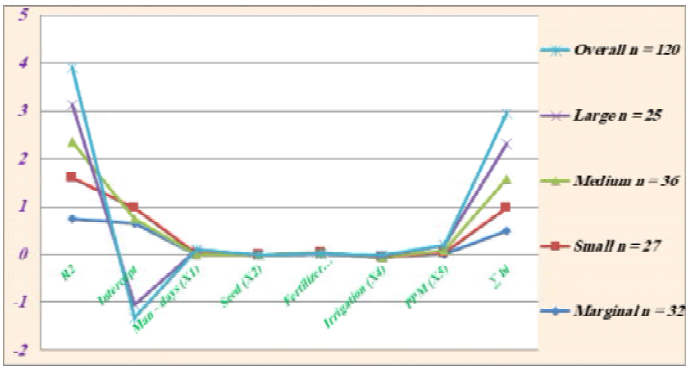

Fig. 1: Estimation of Regression Coefficients of the Chickpea Production

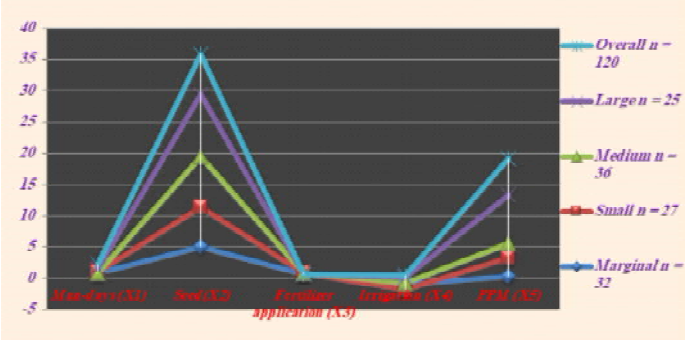

Fig. 2: Estimation of Marginal Value Profitability (MVP) of the Chickpea Production

Table 2: Estimation of Marginal Value Productivity (MVP) of Chickpea Production

Items	Size of groups				Overall n = 120
	Marginal n = 32	Small n = 27	Medium n = 36	Large n = 25	
Man-days (X_1)	0.81	0.17	-0.27	0.82	0.71
Seed (X_2)	5.03	6.39	7.97	9.96	6.29
Fertilizer application (X_3)	0.58	0.28	-0.004	-0.24	0.06
Irrigation (X_4)	-1.05	-0.81	1.12	0.73	0.51
PPM (X_5)	0.24	3.05	2.23	7.85	5.81

increase in man-days x_1 will increase the yield 0.11 percent, 1 percent increase in seed x_2 will increase the yield 0.45 percent, and 1 percent increase in the plant protection measure (PPM) x_5 will increase the yield in 0.07 percent and the overall sum of the elasticities indicates that the diminishing returns to scale (Verma *et al.* 2016).

The marginal value productivity of different inputs and their ratio to their respective prices of different sizes of groups are presented in table 02. It revealed that the man-days x_1 (0.81), seed x_2 (5.03), fertilizer application x_3 (0.58), and plant protection measure x_5 (0.24) in marginal size of the group, man-days x_1 (0.17), seed x_2 (6.39), fertilizer application x_3 (0.28) and plant protection measure x_5 (3.05) in small size of the group, seed x_2 (7.97), irrigation x_4 (1.12) and plant protection measure x_5 (2.23) in medium size of the group, man-days x_1 (0.82), seed x_2 (9.96), irrigation x_4 (0.73) and plant protection measure x_5 (7.85) in large size of the group were underutilized on chickpea growers. However, the marginal value productivity of irrigation x_4 (-1.05) in marginal size of the group, irrigation x_4 (-0.81) in small size of the group, man-days x_1 (-0.27), and fertilizer application x_3 (-0.004) in medium size of farms and fertilizer application x_3 (-0.24) in large size of farms were overutilization of inputs and leaving scope for their inefficient use. The man-days x_1 (0.71), seed x_2 (6.29), fertilizer application x_3 (0.06), irrigation x_4 (0.51), and plant protection measure x_5 (5.81) marginal value productivity overall average of the chickpea growers were underutilized on chickpea production in this study area.

CONCLUSION

The resource use efficiency analysis concluded that there is a need for more investment in man-day (X_1), seed (X_2), and plant protection measures (X_3) in different size groups for increasing the level of chickpea production. Further, the marginal value of productivity analysis also suggests a significant scope for raising chickpea production by reallocation of important inputs i.e. man-days, seed, irrigation, fertilizer application, and plant protection

measure. These inputs could affect the chickpea production for efficient allocation of a scarce resource and necessary efforts should be made by the concern with KVKs scientists or extension personnel.

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Online Manuscript No. 97215 recived on 16.3.2022

Manuscript accepted for publication on 16.11.2022

Journal of Eco-friendly Agriculture **18**(1): 55-60. 2023
doi: 10.5958/2582-2683.2023.00010.2

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Genetic mechanism of heterosis and inbreeding depression for grain physical quality traits in elite rice (*Oryza sativa* L.) crosses

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ABSTRACT

In the present study, heterosis and inbreeding depression were studied to understand the type of gene action and to identify the best crosses for hybrid or varietal development. A lesser amount of heterosis was observed for hulling, milling percentages, and kernel length, the chief reason for the non-expression of heterosis may be due to the complete association of the dominant genes in the parents without any over-dominance. Only three hybrids (WGL-32100 x NH-787, RP-Bio-5478-166 x RP-Bio-5478-176, and RP-Bio-5478-185 x -686) exhibited heterosis with insignificant inbreeding depression hence, selection would be effective to improve head rice recovery in these combinations. The best specific crosses with a high amount of heterosis and high *per se* performance (L/B ratio > 3.5) with no inbreeding depression for kernel L/B ratio are MTU 1010 x WGL-32100, MTU 1010 x RP-Bio-5478-270, MTU 1010 x RP-Bio-5478-166, MTU 1010 x RP-Bio-5478-185 and MTU 1010 x NH-787. In these crosses, selection would be highly effective due to the prevalence of additive gene effects.

***Key words*:** Rice, heterosis, inbreeding depression, additive gene action

The magnitude of heterosis for yield, yield components, and quality traits depends to a larger extent on genetic variation, genetic base, and adaptability of parents. The presence of a significant amount of non-additive gene action is a prerequisite for the commercial exploitation of heterosis in rice. After the discovery of hybrid vigour in plant hybrids, heterosis has received much interest from plant breeders and successfully exploited to increase crop production. The direction (positive or negative) and magnitude of heterosis in the desired direction are of paramount importance in deciding the practical and economical feasibility of the heterosis breeding programme.

Depending upon the breeding objectives, both positive and negative heterosis is useful for crop improvement. In general, positive heterosis is desired for yield, and negative heterosis for early maturity. Heterosis is expressed in three ways, depending on the criteria used to compare the performance of a hybrid. The three ways are mid-parent, standard variety, and better parent heterosis. However, from the plant breeder's viewpoint, a better parent and/or standard variety is more effective. The former is designated as heterobeltiosis (Fanseco and Peterson, 1968) and the latter as standard heterosis (Virmani, 1994). From a practical point of view, standard heterosis is most important because it is aimed at developing desired hybrids superior to the existing high-yielding commercial varieties.

Inbreeding depression (ID) is defined as the lowered fitness or vigour of inbred individuals compared with their non-inbred counterparts. Its converse is heterosis, the 'hybrid vigour' manifested as increased size, growth rate or other parameters resulting from the increase in heterozygosity in F_1 generation crosses between inbred lines. Inbreeding depression, the depressive effect, is the expression of traits arising from increasing homozygosity (Allard, 1960). In quantitative genetics theory, inbreeding depression and heterosis are due to non-additive gene action and are considered to be two aspects of the same phenomenon (Mather and Jinks, 1982). Li *et al.* (1997) suggested that hybrid breakdown in rice was part of inbreeding depression largely related to additive epistasis. In the present study, heterosis and inbreeding depression were studied to understand the type of gene action and to identify the best crosses for hybrid or varietal development.

MATERIALS AND METHODS

The present investigation was carried out at the Regional Agricultural Research Station, Warangal, which is located at an altitude of 304 M above MSL, 17.97° N latitude and 79.60° E longitude during two years 2014-15 and 2015-16 utilizing both *Kharif* and *Rabi* crop seasons in each year. After the crosses were effected during *kharif* 2014, in the next *rabi* 2014-15 itself, all the 45 F_1s without reciprocals along with parents and two check varieties were grown for producing sufficient F_2 seed. The material (Parents, F_1s and F_2s) was planted in randomized block design replicating thrice during the final season *Kharif* 2015 in a separate plot for studying combining ability, heterosis, and inbreeding depression. Parents, hybrids, and check varieties were planted in one row of 3.0 m in length adopting a spacing of 20 cm between the row and 15 cm between the plants within a row with a single seedling per hill. Each entry of F_2 was planted with the same spacing maintaining 120 plants for cross in each replication (8 rows).

The Heterosis was estimated on mid value, a better parent, and also on standard check *viz.,* BPT 5204 for grain physical quality traits.

Test of significance of heterosis

To test the significance for different types of heterosis needs computation of standard error (S.Em.). For relative heterosis and heterobeltiosis, S.Em. were calculated based on error mean squares (EMS) from the ANOVA tables consisting of parents and crosses. The significance of the heterosis and inbreeding depression was tested with 't' test values as per formulae and furnished in Tables 1 to 3.

The significance of heterosis *viz.,* relative/average heterosis, heterobeltiosis, and standard heterosis was then tested by comparing the calculated 't'- value with the tabulated student's 't'-value for appropriate error degrees of freedom at 5 per cent and 1 per cent level of significance (0.05 and 0.01 level of probability), respectively.

RESULTS AND DISCUSSION

Significant heterosis was not observed for hulling and milling characters on mid-parent value and better parents. However, with respect to milling percentage, heterosis was observed on standard check variety BPT 5204. Both positive and negative heterosis was observed on the mid-parent, whereas, the heterosis on the better parent for these characters was almost on the negative side with one or two exceptions. Heterobeltiosis was observed on the positive side, though not at a significant level in 10 cross combinations in the case of hulling percentage, whereas, for milling percentage, six crosses registered heterobeltiosis. Significant positive standard heterosis, heterobeltiosis, and relative heterosis for milling percentage were reported by Begum *et al.* (2020) and Devi *et al.* (2018). The chief reason for the non-expression of heterosis may be due to the complete association of the dominant genes in the parents without any overdominance. Venkanna *et al.* (2014) also noticed negative heterosis over the better parent in six cross combinations at a significant level out of 36 crosses evaluated for hulling percentage, further they did not report significant positive heterosis on the better parent as in the case of our study. Sarial (2014) also did not notice heterosis for both hulling and milling percentages. As there was no significant heterosis, corresponding inbreeding depression was also not significant (Table 1).

For head rice recovery, out of the 45 F_1 hybrids evaluated, only three hybrids (WGL-32100 x NH-787, RP-Bio-5478-166 x RP-Bio-5478-176 and RP-Bio-5478-185 x -686) exhibited heterosis at a significant level on the better parent whereas, on the mid parent, five hybrids were better. The study also revealed that the percentage of heterobeltiosis ranged from 15 to 16% maximum (Table 2). On the standard check, it was on the negative side. Gnanamalar and Vivekanandhan (2013) observed heterosis on the better parent for head rice recovery in certain cross combinations studied unlike in the present study. As the inbreeding depression is not significant in these superior three crosses, it is presumed that additive gene action played important role in the inheritance of this quantitative trait. Hence, selection would be effective to improve head rice recovery in these combinations.

Heterobeltiosis was not observed at a significant level in any of the cross-combinations evaluated for kernel length (Table 2). However, 10 hybrids exhibited significant positive heterosis on the mid-parent. With respect to standard heterosis, mostly it was on the negative side, with only seven crosses with expression at a significant level on the desirable side. These findings are in accordance with the observations of Gnanamalar and Vivekanandhan (2013), Raju *et al.* (2005), Rajendar Reddy *et al.* (2013), Devi *et al.* (2017) and Vadivel *et al.* (2018). Lack of heterosis on the better parent for kernel length as was revealed by earlier workers is ascribed due to a lack of over dominance or complete dominance for expression of this particular trait. Nevertheless, the existence of heterosis over mid-parent without any inbreeding depression offers scope for isolation of homozygous lines with higher kernel length, especially in the case of the cross MTU 1010 x RP-Bio-5478-185. For kernel breadth, heterosis on better and mid-parents is desirable on the negative side for kernel breadth. Heterosis was observed mostly on the negative side on the mid-parent, however, on the better parent, the heterosis on the negative side is low. Though the heterosis was not observed at significant levels, the

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00010.2

Table 1. Estimation of heterosis over mid-parent (H_1) better parent (H_2), standard check (SH) and inbreeding depression (ID) for hulling percent and milling percent

Crosses	Hulling percent					Milling percent				
	Mean	H_1	H_2	SH	ID	Mean	H_1	H_2	SH	ID
MTU-1010 x WGL-32100	78.12	-2.14	-2.61	2.33	2.59	70.43	-3.03	-5.87	8.87**	4.29
MTU-1010 x Ramappa	78.47	-1.92	-2.17	2.79	5.90	70.39	-4.61	-5.92	8.81**	5.94
MTU-1010 x RP-Bio-5478-270	75.41	-4.51	-5.98	-1.22	1.87	68.30	-5.13	-8.71	5.58**	-1.33
MTU-1010 x RP-Bio-5478-166	79.38	0.25	-1.03	3.98	9.30	71.05	-1.42	-5.04	9.83**	7.42
MTU-1010 x RP-Bio-5478-176	76.77	-2.69	-4.29	0.56	4.00	70.46	-3.05	-5.83	8.92**	4.23
MTU-1010 x DRR Dhan - 40	78.46	1.28	-2.18	2.78	3.14	69.53	-2.5	-7.07	7.48**	2.46
MTU-1010 x RP-Bio-5478 -185	76.26	-3.73	-4.92	-0.10	-3.86	68.71	-4.56	-8.17	6.21**	-0.54
MTU-1010 x NH-686	77.76	-2.32	-3.05	1.86	3.29	69.40	-3.75	-7.24	7.28**	7.54
MTU-1010 x NH-787	76.59	-2.98	-4.51	0.33	2.60	69.87	-3.71	-6.62	8.01**	11.06
WGL-32100 x Ramappa	75.96	-4.6	-4.81	-0.50	-2.29	68.27	-4.66	-6.18	5.53**	0.56
WGL-32100 x RP-Bio-5478-270	78.03	-0.72	-1.79	2.21	3.63	70.12	0.45	-0.45	8.39**	7.10
WGL-32100 x RP-Bio-5478-166	73.41	-6.84	-7.6	-3.84	-2.98	67.79	-2.99	-3.76	4.79	0.07
WGL-32100 x RP-Bio-5478-176	79.32	1.03	-0.16	3.90	4.82	70.74	0.35	0.28	9.35**	2.01
WGL-32100 x DRR Dhan - 40	78.03	1.22	-1.79	2.21	7.01	70.91	2.59	0.67	9.62**	3.53
WGL-32100 x RP-Bio-5478 -185	75.72	-3.95	-4.69	-0.81	-3.01	70.24	0.62	-0.28	8.58**	-2.65
WGL-32100 x NH- 686	78.12	-1.39	-1.67	2.33	0.60	70.43	0.74	-0.01	8.87**	-2.71
WGL-32100 x NH- 787	78.47	-0.12	-1.23	2.79	2.63	70.39	0.02	-0.07	8.81**	9.73
Ramappa x RP-Bio-5478-270	75.41	-4.27	-5.5	-1.22	1.11	68.30	-3.76	-6.14	5.58*	2.55
Ramappa x RP-Bio-5478-166	79.38	0.51	-0.53	3.98	0.35	71.05	0.01	-2.36	9.83**	1.37
Ramappa x RP-Bio-5478-176	76.77	-2.43	-3.8	0.56	1.39	70.46	-1.67	-3.17	8.92**	10.43
Ramappa x DRR Dhan - 40	78.46	1.55	-1.68	2.78	3.53	69.53	-1.07	-4.45	7.48**	1.42
Ramappa x RP-Bio-5478 -185	76.26	-3.48	-4.44	-0.10	0.34	68.71	-3.18	-5.58	6.21**	-0.98
Ramappa x NH-686	77.76	-2.07	-2.56	1.86	0.85	69.40	-2.36	-4.63	7.28**	2.25
Ramappa x NH-787	76.59	-2.73	-4.02	0.33	0.64	69.87	-2.33	-3.99	8.01**	0.43

Table 1. (Contd.)

Crosses	Hulling percent					Milling percent				
	Mean	H_1	H_2	SH	ID	Mean	H_1	H_2	SH	ID
RP-Bio-5478-270 x RP-Bio-5478-166	75.96	-2.55	-2.8	-0.50	2.32	68.27	-1.41	-1.51	5.53*	-2.64
RP-Bio-5478-270 x RP-Bio-5478-176	78.03	0.48	0.37	2.21	-1.88	70.12	0.38	-0.6	8.39**	-2.88
RP-Bio-5478-270 x DRR Dhan - 40	73.41	-3.71	-5.57	-3.84	6.28	67.79	-1.01	-2	4.79	-5.69
RP-Bio-5478-270 x RP-Bio-5478 -185	79.32	1.72	1.41	3.90	-0.98	70.74	2.27	2.27	9.35**	2.22
RP-Bio-5478-270 x NH-686	78.03	-0.43	-1.23	2.21	0.04	70.91	2.35	2.19	9.62**	0.93
RP-Bio-5478-270 x NH-787	75.72	-2.56	-2.6	-0.81	1.90	70.24	0.72	-0.1	8.58**	0.00
RP-Bio-5478-166 x RP-Bio-5478-176	73.49	-5.61	-5.96	-3.73	2.57	66.43	-5.01	-5.83	2.69	-2.14
RP-Bio-5478-166 x DRR Dhan - 40	77.81	1.79	-0.44	1.93	2.84	70.10	2.25	1.13	8.36**	-1.93
RP-Bio-5478-166 x RP-Bio-5478 -185	79.39	1.54	1.5	4.00	2.44	72.57	4.8	4.69	12.18**	4.78
RP-Bio-5478-166 x NH- 686	76.82	-2.23	-2.76	0.63	2.37	69.47	0.17	0.12	7.39**	-0.10
RP-Bio-5478-166 x NH-787	76.93	-1.26	-1.56	0.77	0.95	71.66	2.64	1.92	10.77**	1.93
RP-Bio-5478-176 x DRR Dhan - 40	76.00	-0.2	-2.02	-0.45	1.71	70.77	2.31	0.33	9.40**	4.93
RP-Bio-5478-176 x RP-Bio-5478 -185	76.31	-2.03	-2.44	-0.04	0.28	69.32	-0.77	-1.73	7.16**	2.58
RP-Bio-5478-176 x NH-686	78.02	-0.34	-1.24	2.20	-2.81	69.54	-0.61	-1.42	7.50**	0.24
RP-Bio-5478-176 x NH-787	74.97	-3.42	-3.49	-1.79	-2.57	68.92	-2.14	-2.3	6.54**	-1.76
DRR Dhan - 40 x RP-Bio-5478 -185	79.71	4.23	1.9	4.41	1.77	72.33	5.61	4.57	11.81**	-0.03
DRR Dhan - 40 x NH-686	77.09	0.29	-2.42	0.98	0.12	70.42	2.66	1.48	8.86**	3.51
DRR Dhan - 40 x NH-787	81.21	6.57	4.54	6.38	4.94	72.03	4.31	2.45	11.35**	3.93
RP-Bio-5478 -185 x NH-686	74.82	-4.82	-5.29	-1.99	2.70	68.84	-0.64	-0.79	6.42**	2.00
RP-Bio-5478 -185 x NH-787	75.27	-3.44	-3.77	-1.40	0.08	69.94	0.29	-0.53	8.12**	-0.29
NH-686 x NH-787	76.57	-2.26	-3.08	0.30	1.27	68.95	-1.29	-1.93	6.59**	-0.86

*Significant at 5% level, **Significant at 1% level

Table 2. Estimation of heterosis over mid-parent (H_1) better parent (H_2), standard check (SH) and inbreeding depression (ID) for head Rice recovery and kernel length

Crosses	Head rice recovery percent					Kernel length (mm)				
	Mean	H_1	H_2	SH	ID	Mean	H_1	H_2	SH	ID
MTU-1010 x WGL-32100	54.80	0.36	-1.44	-11.33**	-1.09	6.22	6.05	0.65	8.74**	-1.45
MTU-1010 x Ramappa	61.44	11.36	10.5	-0.58	8.35	6.28	4.93	1.62	9.79**	2.07
MTU-1010 x RP-Bio-5478-270	56.85	6.39	2.25	-8.01**	-3.10	6.37	20.3**	3.07	11.36**	-4.55
MTU-1010 x RP-Bio-5478-166	53.73	1.43	-3.36	-13.06**	0.99	6.37	16.35**	3.07	11.36**	2.83
MTU-1010 x RP-Bio-5478-176	59.42	10.85	6.87	-3.85	-0.47	5.70	6.64	-7.77	-0.35	-2.81
MTU-1010 x DRR Dhan - 40	54.50	2.6	-1.98	-11.81**	0.55	6.34	18.84**	2.59	10.84**	-4.26
MTU-1010 x RP-Bio-5478 -185	57.33	8.33	3.11	-7.23*	0.92	6.64	27.45**	7.44	16.08**	-0.75
MTU-1010 x NH-686	59.03	9.51	6.17	-4.48	-1.03	5.72	4.47	-7.44	0.00	0.17
MTU-1010 x NH-787	57.13	7.47	2.75	-7.56*	-1.35	6.39	17.9**	3.40	11.71**	4.54
WGL-32100 x Ramappa	54.46	0.53	-0.51	-11.88**	-5.03	5.89	3.88	1.73	2.97	2.55
WGL-32100 x RP-Bio-5478-270	51.13	-2.5	-4.63	-17.27**	3.38	5.72	14.86*	3.06	0.00	-0.87
WGL-32100 x RP-Bio-5478-166	52.53	1.06	-2.01	-15.00**	3.29	5.24	1.55	-5.59	-8.39**	-1.91
WGL-32100 x RP-Bio-5478-176	58.27	10.76	8.69	-5.71	-3.48	5.46	8.55	-1.62	-4.55*	2.56
WGL-32100 x DRR Dhan - 40	54.88	5.29	2.37	-11.20**	4.99	5.81	15.74**	4.68	1.57	-0.52
WGL-32100 x RP-Bio-5478 -185	54.90	5.73	2.41	-11.17**	2.95	5.24	7.05	-5.59	-8.39	1.91
WGL-32100 x NH- 686	54.80	3.57	2.22	-11.33**	-14.05	5.64	9.3	1.62	-1.40	2.84
WGL-32100 x NH- 787	61.44	17.78*	14.61*	-0.58	5.40	5.80	13.61*	4.50	1.40	0.52
Ramappa x RP-Bio-5478-270	56.85	7.25	3.85	-8.01**	5.58	5.26	3.14	-9.15	-8.04**	1.52
Ramappa x RP-Bio-5478-166	53.73	2.26	-1.85	-13.06**	-3.29	5.69	7.77	-1.73	-0.52	1.23
Ramappa x RP-Bio-5478-176	59.42	11.74	8.55	-3.85	5.07	5.83	13.2*	0.69	1.92	-0.51
Ramappa x DRR Dhan - 40	54.50	3.44	-0.44	-11.81**	-3.63	5.16	0.39	-10.88	-9.79	0.19
Ramappa x RP-Bio-5478 -185	57.33	9.22	4.73	-7.23**	5.60	5.74	14.46*	-0.86	0.35	3.31
Ramappa x NH-686	59.03	10.39	7.84	-4.48	6.54	5.27	-0.19	-8.98	-7.87**	3.04
Ramappa x NH-787	57.13	8.34	4.37	-7.56*	1.14	5.81	11.2	0.35	1.57	-0.17

Table 2 : (Contd.)

Crosses	Head rice recovery percent					Kernel length (mm)				
	Mean	H_1	H_2	SH	ID	Mean	H_1	H_2	SH	ID
RP-Bio-5478-270 x RP-Bio-5478-166	54.46	7.18	6.22	-11.88**	0.46	4.18	-8.93	-12.37	-26.92**	8.13
RP-Bio-5478-270 x RP-Bio-5478-176	51.13	-0.6	-0.93	-17.27*	-19.11	4.07	-8.74	-9.76	-28.85**	0.25
RP-Bio-5478-270 x DRR Dhan - 40	52.53	3.09	2.46	-15.00**	-13.84	4.35	-2.25	-3.12	-23.95**	4.14
RP-Bio-5478-270 x RP-Bio-5478 -185	58.27	14.81	13.65	-5.71	1.65	4.31	-0.35	-2.27	-24.65**	-7.19
RP-Bio-5478-270 x NH-686	54.88	6.07	5.11	-11.20**	-6.45	4.07	-11.33	-14.68*	-28.85**	2.95
RP-Bio-5478-270 x NH-787	54.90	7.66	7.08	-11.17**	-7.65	4.13	-8.93	-11.37	-27.80**	-3.63
RP-Bio-5478-166 x RP-Bio-5478-176	59.69	17.09*	15.66*	-3.41	4.51	4.29	-7.54	-10.06	-25.00**	-9.32
RP-Bio-5478-166 x DRR Dhan - 40	51.34	1.67	1.38	-16.93**	-6.35	4.47	-3.46	-6.29	-21.85**	-8.28
RP-Bio-5478-166 x RP-Bio-5478 -185	58.79	16.89*	16.76*	-4.87	4.24	4.45	-1.22	-6.71	-22.20**	-3.60
RP-Bio-5478-166 x NH- 686	59.25	15.54	13.48	-4.13	-0.08	4.13	-13.42*	-13.42	-27.80**	2.91
RP-Bio-5478-166 x NH-787	56.09	10.99	10.59	-9.24**	-5.90	4.35	-7.74	-8.81	-23.95**	-9.20
RP-Bio-5478-176 x DRR Dhan - 40	55.73	9.01	7.98	-9.82**	-7.02	4.13	-8.22	-8.43	-27.80**	-3.63
RP-Bio-5478-176 x RP-Bio-5478 -185	56.02	10	8.54	-9.35**	3.23	4.14	-5.37	-8.2	-27.62**	0.48
RP-Bio-5478-176 x NH-686	57.21	10.21	9.58	-7.43**	0.02	4.33	-6.68	-9.22	-24.30**	2.54
RP-Bio-5478-176 x NH-787	58.18	13.71	12.73	-5.86	-0.09	4.27	-6.87	-8.37	-25.35**	3.28
DRR Dhan - 40 x RP-Bio-5478 -185	51.40	1.90	1.5	-16.83*	-3.52	4.35	-0.34	-3.12	-23.95**	-0.23
DRR Dhan - 40 x NH-686	58.85	14.44	12.72	-4.77	2.23	4.22	-8.86	-11.53	-26.22**	4.03
DRR Dhan - 40 x NH-787	50.97	0.57	0.49	-17.52**	-4.43	4.15	-9.29	-10.94	-27.45**	-2.89
RP-Bio-5478 -185 x NH-686	60.28	17.68*	15.46*	-2.46	3.43	4.43	-1.66	-7.13	-22.55**	-0.90
RP-Bio-5478 -185 x NH-787	52.91	4.81	4.32	-14.39**	-4.40	4.40	-1.12	-5.58	-23.08**	5.00
NH-686 x NH-787	55.61	8.05	6.51	-10.02**	-0.47	4.31	-8.59	-9.64	-24.65**	-1.62

*Significant at 5% level, **Significant at 1% level

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00010.2

Table 3. Estimation of heterosis over mid-parent (H_1) better parent (H_2), standard check (SH) and inbreeding depression (ID) for kernel breadth and kernel L/B ratio

Crosses	Kernel breadth (mm)					Kernel L/B ratio (mm)				
	Mean	H_1	H_2	SH	ID	Mean	H_1	H_2	SH	ID
MTU-1010 x WGL-32100	1.79	-12.04	-7.25	-15.17**	-1.12	3.47	19.47**	8.11*	27.57**	-0.58
MTU-1010 x Ramappa	1.95	-1.52	1.04	-7.58	1.54	3.22	6.44	0.52	18.38**	0.62
MTU-1010 x RP-Bio-5478-270	1.79	-6.28	-5.29	-15.17**	-7.26	3.56	28.92**	11.23**	30.88**	2.53
MTU-1010 x RP-Bio-5478-166	1.77	-11.72	-8.29	-16.11**	6.21	3.60	30.87**	12.37**	32.35**	-3.33
MTU-1010 x RP-Bio-5478-176	2.09	6.63	8.29	-0.95	-6.22	2.72	-0.55	-15.07**	0.00	2.94
MTU-1010 x DRR Dhan - 40	1.96	0.00	1.55	-7.11	5.61	3.23	18.59**	1.14	18.75**	-10.53
MTU-1010 x RP-Bio-5478 -185	1.89	-4.30	-2.07	-10.43	-11.64	3.55	33.71**	10.71**	30.51**	10.70
MTU-1010 x NH-686	2.04	-1.21	5.70	-3.32	2.45	2.80	4.09	-12.68**	2.94	-2.50
MTU-1010 x NH-787	1.86	-7.23	-3.63	-11.85*	-3.23	3.44	26.36**	7.38*	26.47**	7.85
WGL-32100 x Ramappa	2.04	-2.16	0.49	-3.32	0.49	2.88	5.63	0.94	5.88	1.39
WGL-32100 x RP-Bio-5478-270	2.10	4.22	11.11	-0.47	-6.19	2.73	10.77**	5.01	0.37	5.13
WGL-32100 x RP-Bio-5478-166	2.02	-4.27	-2.88	-4.27	-2.97	2.60	6.06	0.00	-4.41	1.15
WGL-32100 x RP-Bio-5478-176	2.01	-2.66	1.01	-4.74	-3.98	2.71	11.51**	4.49	-0.37	5.90
WGL-32100 x DRR Dhan - 40	2.12	2.66	6.53	0.47	4.25	2.74	12.89**	5.65	0.74	-5.11
WGL-32100 x RP-Bio-5478 -185	2.02	-2.88	0.00	-4.27	2.97	2.59	10.07*	-0.39	-4.78	-1.54
WGL-32100 x NH- 686	2.06	-5.07	-3.74	-2.37	-5.34	2.74	14.88**	5.52	0.74	8.03
WGL-32100 x NH- 787	2.04	-3.32	-1.92	-3.32	5.88	2.84	17.22**	9.24*	4.41	-5.99
Ramappa x RP-Bio-5478-270	1.94	-1.02	2.65	-8.06	-3.09	2.70	4.44	-5.15	-0.74	4.07
Ramappa x RP-Bio-5478-166	1.97	-4.14	-2.96	-6.64	-4.06	2.88	11.97**	1.17	5.88	4.86
Ramappa x RP-Bio-5478-176	2.09	3.98	5.03	-0.95	-0.48	2.78	8.59*	-2.46	2.21	-0.72
Ramappa x DRR Dhan - 40	1.97	-1.99	-1.01	-6.64	3.05	2.62	2.48	-8.07*	-3.68	-2.67
Ramappa x RP-Bio-5478 -185	2.03	0.25	0.5	-3.79	-1.97	2.83	14.13**	-0.82	4.04	5.30
Ramappa x NH-686	1.97	-6.86	-2.96	-6.64	1.02	2.68	6.83	-5.85	-1.47	2.24
Ramappa x NH-787	2.12	3.16	4.43	0.47	4.25	2.73	7.33	-4.09	0.37	-5.13

Table 3. (Contd.)

Crosses	Kernel breadth (mm)					Kernel L/B ratio (mm)				
	Mean	H_1	H_2	SH	ID	Mean	H_1	H_2	SH	ID
RP-Bio-5478-270 x RP-Bio-5478-166	1.73	-12.85	-8.47	-18.01**	9.83	2.42	4.76	4.15	-11.03*	-2.07
RP-Bio-5478-270 x RP-Bio-5478-176	1.75	-9.79	-7.41	-17.06**	-8.57	2.32	1.09	-0.14	-14.71**	8.19
RP-Bio-5478-270 x DRR Dhan - 40	1.95	0.52	3.17	-7.58	-8.72	2.23	-2.83	-4.15	-18.01**	11.66
RP-Bio-5478-270 x RP-Bio-5478 -185	1.80	-7.93	-4.76	-14.69*	-5.00	2.39	7.90	2.72	-12.13**	-1.67
RP-Bio-5478-270 x NH-686	1.86	-9.05	-1.59	-11.85*	11.83	2.19	-2.67	-5.87	-19.49**	-10.05
RP-Bio-5478-270 x NH-787	1.76	-11.34	-6.88	-16.59**	-2.84	2.35	2.84	1.00	-13.60**	-1.28
RP-Bio-5478-166 x RP-Bio-5478-176	1.88	-7.62	-5.53	-10.90*	-11.70	2.28	-0.07	-0.72	-16.18**	1.75
RP-Bio-5478-166 x DRR Dhan - 40	2.01	-1.23	1.01	-4.74	-1.49	2.22	-2.7	-3.48	-18.38**	-7.21
RP-Bio-5478-166 x RP-Bio-5478 -185	2.00	-2.44	-0.99	-5.21	-5.50	2.22	0.68	-3.62	-18.38**	1.80
RP-Bio-5478-166 x NH- 686	1.96	-8.41	-5.77	-7.11	12.24	2.12	-5.37	-7.97	-22.06**	-10.38
RP-Bio-5478-166 x NH-787	2.24	7.69	7.69	6.16	-8.93	1.94	-14.75**	-15.8**	-28.68**	0.00
RP-Bio-5478-176 x DRR Dhan - 40	1.99	0.00	0.00	-5.69	-3.02	2.05	-9.41*	-9.54	-24.63*	-1.95
RP-Bio-5478-176 x RP-Bio-5478 -185	2.07	3.24	4.02	-1.90	-13.04	2.01	-7.93	-11.31*	-26.10**	12.44
RP-Bio-5478-176 x NH-686	2.07	-1.19	4.02	-1.90	-5.80	2.09	-5.78	-7.78	-23.16**	8.13
RP-Bio-5478-176 x NH-787	2.06	1.23	3.52	-2.37	-9.22	2.08	-7.98	-8.52	-23.53**	11.54
DRR Dhan - 40 x RP-Bio-5478 -185	2.00	-0.25	0.50	-5.21	-3.50	2.17	-0.46	-3.98	-20.22**	2.76
DRR Dhan - 40 x NH-686	1.91	-8.83	-4.02	-9.48	10.99	2.22	0.08	-1.91	-18.38**	-7.21
DRR Dhan - 40 x NH-787	1.81	-11.06	-9.05	-14.22*	-11.05	2.31	2.37	1.91	-15.07*	7.79
RP-Bio-5478 -185 x NH-686	2.18	3.32	7.92	3.32	5.96	2.04	-4.75	-6.29	-25.00**	-6.86
RP-Bio-5478 -185 x NH-787	2.04	-0.49	0.99	-3.32	1.47	2.15	-0.92	-4.01	-20.96**	3.72
NH-686 x NH-787	2.06	-3.74	-0.96	-2.37	-15.05	2.09	-5.21	-6.69	-23.16**	11.48

*Significant at 5% level, **Significant at 1% level, H_1: Average heterosis; H_2 : Heterobeltiosis; SH: Standard heterosis; ID: Inbreeding depression

doi: 10.5958/2582-2683.2023.00010.2

magnitudes were highly variable with the highest desirable heterosis in the case of cross combination MTU 1010 x WGL-32100, MTU 1010 x RP-Bio-5478-166, DRR Dhan 40 x NH-787. On standard check variety, 11 crosses expressed desirable heterosis at a significant level with a maximum percentage in case RP-Bio-5478-270 x RP-Bio-5478-166, RP-Bio-5478-270 x RP-Bio-5478-176, RP-Bio-5478-270 x NH-787, MTU 1010 x RP-Bio-5478-166 (Table 3). In these crosses, the inbreeding depression was observed on the desirable side, therefore, selection would be highly effective for the development of slender-grained genotypes.

Among the dimensions of Kernel, L/B ratio is treated as the most important one from the point of trade. Generally, lower levels of heterosis persist for this quantitative trait. In the present study, 14 cross combinations on mid-parent and five cross combinations on better parent registered the heterosis that too with highly varying levels. Significant positive heterosis for L/B ratio was also reported by Yadav *et al.* (2004), Eradasappa *et al.* (2007), and Varpe *et al.* (2011). Significant inbreeding depression for Hulling (%), milling (%), and L/B ratio was reported by Adilakshmi and Raghava Reddy (2011). The best specific crosses with a high amount of heterosis and high *per se* performance (L/B ratio > 3.5) with no inbreeding depression are MTU 1010 x WGL-32100, MTU 1010 x RP-Bio-5478-270, MTU 1010 x RP-Bio-5478-166, MTU 1010 x RP-Bio-5478-185 and MTU 1010 x NH-787 (Table 3).

Hence, it can be concluded that in these crosses, selection would be highly effective due to the prevalence of additive gene effects. whereas, Raju *et al.* (2005) and Venkanna *et al.* (2014) observed heterosis followed by inbreeding depression and were of the opinion that recurrent selection and bi-parental matings would be more effective in view of the existence of non-additive genetic effects. WGL-32100 x NH-787, RP-Bio-5478-166 x Rp-Bio-5478-176 and RP-Bio-5478-185 x NH-686 for HRR percentage, MTU 1010 x RP-Bio-5478-166, RP-Bio-5478-270 x RP-Bio-5478-166 and RP-Bio-5478-270 x NH-787 for kernel breadth, MTU 1010 x WGL-32100, MTU 1010 x RP-Bio-5478-270, MTU 1010 x RP-Bio-5478-166, MTU 1010 x RP-Bio-5478-185 for kernel L/B ration were identified as the top ranking crosses for selection in segregating generations on account of high *per se* with high heterosis followed by low inbreeding depression indicating the role of additive gene action.

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Manuscript recived on 09.08.2022
Manuscript accepted for publication on 13.11.2022

Journal of Eco-friendly Agriculture **18**(1): 61-67. 2023
doi: 10.5958/2582-2683.2023.00011.4

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Determination of combining ability and gene effects for yield and yield attributes in Sesame (*Sesamum indicum* L.)

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ABSTRACT

To identify combiners and developing cross combinations to exploit heterosis, a set of 10 diverse parental lines were crossed in half-diallel mating design to study the combining ability and gene action for yield and yield attributes. 45 F_1's along with ten parents and one check were evaluated for twelve traits in randomized block design with two replications *viz*., days to 50 percent flowering, days to maturity, plant height, height to fight capsule, number of branches per plant, number of capsules on main stem, number of capsules per plant, length of capsule, number of seeds per capsule, 1000 seed weight, oil content and seed yield per plant. Analysis of variance and the combining ability showed significant variation among parents, hybrids and parents vs hybrids. Combining ability analysis revealed importance of non-additive genetic variances for the control of all traits. From the evaluation of gca effects in parents, it was observed that DS-5 and RMT-430 is a best general combiner for most of the traits followed by IC-204845. Based on *per se* performance and sca effects of the hybrid crosses JTS-8 X JLT 408, TKG-22 X RT 368, JTS-8 X MT-10-8-1, RMT-430 X MT-10-8-1 and DS-5 X DSS-9 were identified to be top promising five heterotic hybrids for seed yield. While the hybrids TKG-22 X AKT-101, DS-5 X RT-368, IC-204845 X AKT-101, TKG-22 x RT-368 and DSS-9 x JTS-8 were identified for oil content and these crosses can be best exploited for the improvement of oil content in Sesame.

Keywords: Sesame, Combining ability, Gene action, *Per se* performance, Yield attributes

Sesame (*Sesamum indicum* L.) belonging to the order *Tubiflorae* and family *pedaliaceae* is probably the most ancient oilseed known with longest history of cultivation in India and its domestication is lost in the mists of antiquity (Weiss, 1983). It is often referred as the epithet "Queen of oilseeds by virtue of quality of oil it produces. Sesame seed contains oil (37-63%), protein (22-24%), carbohydrates (10-12%), and crude fiber (4-7%) (Makinde and Akinoso, 2013). It is rich in unsaturated fatty acids, methionine, and tryptophan. Also, rich in micronutrients such as minerals, lignans, tocopherol, and phytosterol (Hassan *et al*. 2018).

World sesame area was 105.70 lakh hectares with a production of 61.1 lakh tonne and a productivity of 535 kg ha^{-1}. India is a global leader in sesame production. Sesame is grown in India on an area of 15.66 lakh hectares with 7.43 lakh tonne of production. In Karnataka, it is grown on an area of 0.35 lakh hectares with a production of 0.22 lakh tonne and an average yield of 629 kg / ha (Anon, 2017-18). In India, however the productivity is poor compared to other countries that need improvement. Most of the varieties developed and commercialized for cultivation are under low management rates selections from local or closely related populations.

Further, an understanding of the combining ability and gene action is a prerequisite for any successful plant breeding programme. Testing the parents for their combining ability is very important because many times, the high yielding parents may not do well to give good hybrids. Combining ability is an efficient tool which helps in the identification of desirable parents. The selected good combiner could be later utilizing in the hybridization programme to come-up with desirable combination of genes, which in turn helps to formulate suitable breeding strategy to develop high yielding genotypes or hybrids with desirable agronomic traits. Diallel analysis helps in testing a large number of genotypes to assess the gene action and combining ability.

The concept of combining ability analysis give precise estimates of the nature and magnitude of gene actions involved in the inheritance of quantitative traits which inturn facilitates the identification of parents with combining ability effects and the cross combinations with good specific combining ability effects. The knowledge on combining

ability and type of gene action helps in selecting the most suitable breeding methods. The present investigation aims to identify the good general combiners and specific cross combinations and to exploit heterosis for increasing the seed yield and its components in sesame.

MATERIALS AND METHODS

The study was carried out at All India Co-ordinated Research Project on Sesame, Main Agriculture Research Station, University of Agricultural Sciences, Dharwad (Karnataka). The experimental plants of 10 morphological diverse genotypes *viz.*, DSS-9, RMT-430, IC-204845, JLT-408, MT-10-8-1, RT-368, AKT-101, DS-5 (standard check), JTS-8 (zonal check) and TKG-22 (national check), and their 45 direct crosses *i.e.*, the F_1 hybrids. All the 55 treatments (10 parents and 45 F_1s) were grown in Randomized Block Design with two replications during *kharif*, 2019 with the objectives to estimate the combining ability and gene action. Observations were recorded on five plants selected at random in each entry in each replication for twelve quantitative traits *viz.*, Days to 50 per cent flowering, Days to maturity, plant height (cm), height to fight capsule (cm), number of branches per plant, number of capsules on main stem, number of capsules per plant, length of capsule (cm), number of seeds per capsule, 1000 seed weight (g), oil content (%) and seed yield per plant (g). The parents and F_1s were sown in a single row of 3m length in each replication with a spacing of 30 cm between the rows and 15 cm between plants. Crop was raised by following recommended Agronomic practices. Variety DS-5 was used as the standard parental check. DS-5 is one of the leading variety which matures in 90-95 days, more number of elongated capsules having white bold seeds and high oil content of 49 to 52 per cent. The data obtained for each character were analyzed by the usual standard statistical procedure i.e., Method of analysis for combining ability was based on method–II, model I as outlined by (Griffing, 1956).

RESULTS AND DISCUSSION

The ANOVA for the treatment mean sum of squares was significant for days to maturity, plant height, number of branches per plant, number of capsules per plant, 1000 seed weight, oil content and seed yield per plant. The variances due to parents and hybrids were highly significant for all the traits. Parents *vs* hybrids showed highly significant differences for days to 50 per cent flowering, days to maturity, plant height, height to fight capsule, number of branches per plant, number of capsules on main stem, number of capsules per plant, length of capsule, number of seeds per capsule, 1000 seed weight, oil content and seed yield per plant (Table 1).

The combining ability analysis suggested that a highly significant interaction existed between parents for all the traits studied. The parents DS-5, RMT-430, TKG-22 and JTS-8 (seed yield); DS-5, MT-10-8-1, JTS-8 and TKG-22 (oil content); RMT-430, DS-5, TKG-22 (1000 seed weight); RMT-430, TKG-22, JTS-8 and DS-5 (number of seeds per capsule); TKG-22, DS-5 and RMT-430 (Capsule length); DS-5, DSS-9 and TKG-22 (number of branches per plant); TKG-22, RMT-430, IC-204845 and MT-10-8-1 (number of capsules on main stem); IC-204845, RMT-430, DSS-9 and TKG-22 (number of capsules per plant); DSS-9, DS-5 and JTS-8 (50% flowering); TKG-22, JTS-8 and DSS-9 (days to maturity); DS-5, RT-368, IC-204845 and JLT-408 (plant height); JTS-8, DS-5, RMT-430 and AKT-101 (height to first capsule) were found to be good general combiners (Table 2).

On the basis of sca effects, the best hybrids were DS-5 x DSS-9 for days to 50 per cent flowering, DS-5 x DSS-9 for days to maturity, TKG-22 x AKT-101 for plant height, RMT-430 x TKG-22 for height to first capsule, RMT-430 x IC-204845 for number of branches per plant, JTS-8 x RMT-430 for number of capsules on main stem, RMT-430 x IC-204845 for number of capsules per plant, RMT-430 x MT-10-8-1 for

Table 1: Mean sum of squares for combining ability in respect of twelve characters in Sesame

Source of variation	d.f	Days to 50 per cent flowering	Days to maturity	Plant height (cm)	Height to first capsule (cm)	No. of branches per plant	No. of capsules on main stem	No. of capsules per plant	Capsule length (cm)	No. of seeds per capsule	1000 seed weight (g)	Oil content (%)	Seed yield per plant (g)
Replication	1	0.01	0.23	0.28	8.18	0.04	0.22	0.06	0.01	0.95	0.02	5.04	0.02
Genotypes	54	14.13**	22.69**	130.98**	42.66**	0.59**	29.08**	198.97**	0.20**	104.34**	0.73**	21.75**	3.57**
Parents	9	12.20**	31.92**	85.28**	39.28**	0.42**	12.27**	188.63**	0.53**	88.17**	0.48**	19.10**	2.89**
Hybrids	44	14.55**	19.72**	141.90**	42.74**	0.64**	32.60**	205.52**	0.14**	106.66**	0.76**	22.56**	3.64**
Parents vs Hybrids	1	12.93**	70.64**	61.27**	69.44**	0.00018	24.99**	3.36	0.09	147.82**	1.68**	9.53	6.44**
Error	54	1.08	2.82	5.35	3.77	0.05	2.84	4.26	0.06	2.55	0.01	3.06	0.01

* - Significant at 5% level **- Significance at 1% level

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00011.4

Table 2: General combining ability (*gca*) effects of parents with respect to twelve characters in Sesame

Parents	Days to 50 per cent flowering	Days to maturity	Plant height (cm)	Height to first capsule (cm)	No. of branches per plant	No. of capsules on main stem	No. of capsules per plant	Capsule length (cm)	No. of seeds per capsule	1000 seed weight (g)	Oil content (%)	Seed yield per plant (g)
DS-5	-0.30	0.20	4.01 **	-0.78*	0.42 **	-0.84 *	-0.16	0.08	1.19 **	0.17 **	1.45**	0.40**
DSS-9	-1.68 **	-0.76 *	0.08	-0.35	0.12*	-0.79 *	1.60 **	-0.06	-1.75 **	-0.05 **	-0.12	-0.08 **
JTS-8	-0.22	-0.80 *	-1.23 **	-1.02**	-0.19 **	-0.17	-3.10 **	0.02	1.36 **	0.02	0.50	0.19**
RMT-430	1.83 **	0.66 *	-1.81 **	-0.72	-0.04	0.91 **	2.72 **	0.08	2.06 **	0.18 **	-0.13	0.27 **
TKG-22	1.83 **	-1.43 **	-2.83 **	-0.27	0.06	1.57 **	1.50 **	0.12 *	1.89 **	0.17 **	0.26	0.44 **
IC-204845	0.50 *	0.03	1.47 **	2.92**	0.01	0.87 **	7.87 **	-0.01	-0.06	-0.03	0.14	-0.02
JLT-408	-0.09	0.58	0.65	-0.07	0.04	-1.26 **	-1.07 **	0.01	-2.87**	-0.22 **	-0.23	-0.11**
MT-10-8-1	0.24	0.58	-2.36 **	0.79*	-0.15 **	0.48	-1.63 **	-0.21 **	0.29	0.07 **	0.75*	0.01
RT-368	0.95 **	0.49	1.95 **	-0.09	-0.14 **	-1.41 **	-0.72	0.03	0.41	0.01	-0.27	-0.31 **
AKT-101	0.12	0.45	0.07	-0.43	-0.13 **	0.64	-7.00 **	-0.06	-2.51**	-0.33 **	-2.32	-0.78 **

* - Significant at 5% level **- Significance at 1% level

capsule length, JTS-8 x JLT-408 for number of seeds per capsule, JTS-8 x JLT-408 for 1000 seed weight, IC 204845 x AKT-101 for oil content, JTS-8 x JLT-408 for seed yield per plant (Table 3).

The ratio of variance components suggested the importance of non-additive gene action for the traits *viz*., days to 50 per cent flowering, days to maturity, plant height, height to first capsule, number of branches per plant, capsule length, number of capsules per plant, number of capsules on main stem, number of seeds per capsule, 1000 seed weight, oil content and seed yield per plant (Table 4).

Combining ability for yield and yield components

Days to maturity directly indicates the duration of the genotype. As compared to late flowering genotypes early flowering ones are physiologically more efficient. For this trait, negative gca effects were noticed in JTS-8, DSS-9 and TKG-22 among parents and DSS-9 and JLT-408 reported good general combiners. The hybrids DSS-9 x JLT-408, RMT-430 x IC-204845 and DS-5 x JLT-408 were found to be early maturing type and these three hybrids reported negative sca effects for this trait. The hybrid DS-5 x JLT-408 has showed the parents with non significant gca combinations.

Generally, total biomass production and seed yield were directly influenced by maturity. For the days to maturity trait, SCA variance was more than GCA variance indicating that days to maturity was largely governed by the non-additive gene action obtained Joshi *et al*. (2015); Vidhyavathi *et al*. (2005); Musibau and Joseph (2014); Saxena *et al*. (2017); Balaram *et al*. (2018) and Parameshwarappa *et al*. (2021). Therefore, to obtain early maturing hybrids, it is advisable to use suitable parents governing negative gca effects.

The important quantitative trait that has a greater influence on the seed yield is the number of capsules per plant. The gca effects of parents indicated that IC-204845, RMT-430, DSS-9 and TKG-22 are good general combiners. While among hybrids, RMT-430 x IC-204845 (18.46) recorded highest sca effects followed by DSS-9 x MT-10-8-1 (16.18), DS-5 X RT-368 (15.63) and JTS-8 x JLT-408 (12.22) (Table 3). The hybrid RMT-430 x IC-204845 also exhibited significant positive heterosis and its parents involved in this cross exhibited significant high gca effects.

Number of capsules is an important yield contributing trait greatly influenced by the number of branches and plant height. In the present study, it has been found that SCA variance is greater than GCA variance. It reveals the importance of non-additive gene action for inheritance of this trait. Similar results were obtained by Krishnaiah *et al.* (2002); Mothilal and Manoharan (2004); Vidyavathi *et al.* (2005); Saxena *et al*. (2017) and Balaram *et al*. (2018).

The main attribute which has a positive direct association with seed yield is the 1000 seed weight. The gca effects of parents indicated that RMT-430, DS-5 and TKG-22 were good general combiners. High sca effects were recorded in JTS-8 x JLT-408 (1.33) followed by DS-5 x DSS-9 (0.88), RMT-430 x IC-204845 (0.86), DS-5 x RT-368 (0.84) and DSS-9 x IC-204845 (0.84) and also these hybrids expressed positive heterosis for this trait (Table 3). The best hybrid combination JTS-8 x RT-368 has the parents with significant gca effects.

Test weight is one of the important yield contributing character. The SCA variance was higher than the GCA variance, as the ratio of GCA and SCA was less than one indicating the presence of non-additive gene action among hybrids. Vekaria *et al*. (2014) ; Joshi *et al.* (2015); Meenakumari *et al.* (2015) and Balaram *et al*. (2018) reported governing of

Table 3: Specific combining ability effects (*sca)* for hybrids in respect of twelve characters in sesame

Hybrids	Days to 50 per cent flowering	Days to maturity	Plant height (cm)	Height to first capsule (cm)	Number of branches per plant	Number of capsules on main stem	Number of capsules per plant	Capsule length (cm)	Number of seeds per capsule	1000 seed weight (g)	Oil content (%)	Seed yield per plant (g)
DS-5 x DSS-9	6.10 **	8.46 **	2.14	9.46**	-0.13	-2.50 *	-0.48	0.39 *	9.23 **	0.88 **	2.22	1.51 **
DS-5 X JTS-8	1.64 *	3.00 **	-6.15 **	4.23**	0.28	-3.11 **	-6.78 **	-0.28	-10.37 **	-0.87 **	-1.46	-1.86 **
DS -5X RMT-430	1.60 *	1.04	1.74	4.83**	0.32 *	-2.39 *	-6.11 **	-0.20	-4.47 **	-0.92 **	-1.88	-0.51 **
DS-5 X TKG-22	-2.73 **	-0.38	-3.24 *	-4.12**	-0.47 **	3.10 **	3.62 *	0.22	3.59 **	0.12	-4.27**	0.15
DS-5 X IC-204845	-0.06	-0.33	3.55 *	-1.12	0.28	-3.75 **	-4.76 **	0.06	3.64 **	-0.22 **	3.60**	0.44 **
DS-5 X JLT-408	-4.98 **	-4.88 **	0.17	1.68	0.24	5.68 **	-7.32 **	-0.09	1.55	0.64 **	-0.26	0.01
DS-5 X MT-10-8-1	-4.81 **	-3.38 **	11.23 **	5.73**	0.34 *	8.14 **	4.94 **	0.05	-3.71 **	-0.47 **	2.17	-0.36 **
DS-5 X RT-368	0.98	-0.79	4.47 **	-5.70**	0.12	3.33 **	15.63 **	0.52 **	9.27 **	0.84 **	2.38*	1.76 **
DS-5 X AKT-101	-4.19 **	-2.75 *	5.95 **	-6.66**	0.11	-2.42 *	5.72 **	-0.34 *	-5.91 **	-0.41 **	-1.50	-1.07 **
DSS-9 X JTS-8	-1.48 *	3.46 **	1.98	2.80*	0.49 **	-7.17 **	2.76 *	0.02	4.66 **	0.11	1.52	0.44 **
DSS-9 X RMT-430	0.48	-1.50	-0.64	0.30	0.13	1.06	-13.87 **	-0.42 *	-8.84 **	-0.80 **	-3.90**	-2.45 **
DSS-9 X TKG-22	-0.86	-1.42	-5.46 **	2.45	0.14	6.50 **	-0.64	-0.15	-5.57 **	-0.58 **	0.07	-0.88 **
DSS-9 X IC 204845	-2.69 **	-1.38	10.78 **	4.70**	0.09	3.99 **	10.78 **	0.05	1.98	0.84 **	2.78*	0.84 **
DSS-9 X JLT-408	-4.61 **	-7.92 **	-6.45 **	-0.35	0.05	5.43 **	0.83	-0.03	-0.62	-0.07	0.15	0.11
DSS-9 X MT-10-8-1	-1.44 *	-3.42 **	-5.09 **	-2.50	0.44 **	-5.32 **	16.18 **	0.04	-4.47 **	-0.54 **	-3.93**	-0.89 **
DSS-9 X RT-368	2.85 **	6.67 **	1.20	0.37	0.03	-2.12	1.37	0.08	3.40 **	0.75 **	3.21**	1.14 **
DSS-9 X AKT-101	3.69 **	3.21 **	-2.12	-1.90	-0.78 **	-4.77 **	4.96 **	-0.07	-4.27 **	-0.22**	-2.86*	-0.56 **
JTS-8 X RMT-430	-0.48	1.54	-3.23 *	-8.13**	-0.56**	8.34 **	2.73 *	-0.02	-6.44 **	-0.22 **	1.33	-0.25 **
JTS-8 X TKG-22	-1.31	1.13	-0.01	-2.40	-0.06	-0.12	6.26 **	0.25	13.43 **	0.78 **	0.68	1.90 **
JTS-8 X IC 204845	1.85 **	-1.33	-0.21	2.83*	0.59 **	4.78 **	1.58	-0.26	-8.12 **	-0.44 **	0.45	-1.37 **
JTS-8 X JLT-408	2.94 **	-0.88	13.11 **	5.42**	0.86 **	-3.39 **	12.22 **	0.49 **	18.88 **	1.33 **	3.15**	3.73 **
JTS-8 X MT-10-8-1	-0.90	-0.38	1.37	-1.53	-0.05	0.87	-6.52 **	0.44 **	5.13 **	0.67 **	-0.65	0.90 **
JTS-8 X RT-368	-1.11	-4.79 **	0.21	3.14*	-0.26	-2.44 *	-5.63 **	-0.22	-4.30 **	-0.60**	3.61**	-0.97 **
JTS-8 X AKT-101	1.73 *	2.25 *	-2.01	-0.72	-0.47**	1.81	-8.54 **	-0.08	3.23 **	-0.02	0.41	-0.05
RMT-430 X TKG-22	-0.86	-1.83	-11.02 **	-11.38**	-1.01**	-1.30	-19.67**	-0.54**	-14.87 **	-0.81 **	-5.15**	-2.58 **
RMT-430 X IC 204845	-1.69 *	-5.29 **	4.67 **	3.03*	1.04 **	0.40	18.46 **	0.32	8.48 **	0.86 **	-4.53**	1.14 **
RMT-430 X JLT-408	0.39	1.17	-10.71 **	-2.58*	-0.60**	-2.67 *	-0.90	0.15	3.18 **	0.13	3.34**	0.66 **
RMT-430 X MT-10-8-1	1.06	-3.83 **	-5.80 **	-1.23	-0.71**	2.19	-8.74 **	0.53 **	5.43 **	0.68 **	1.40	1.08 **
RMT-430 X RT-368	-1.15	-3.25 **	0.80	4.84**	0.08	1.28	1.45	-0.01	0.60	-0.11	2.54*	0.51 **
RMT-430 X AKT-101	0.69	-1.21	-9.63 **	-1.32	0.37 *	1.73	11.23 **	-0.15	-6.57 **	-0.22 **	0.28	-0.61 **
TKG-22 X IC 204845	1.48 *	-1.21	-14.01 **	-2.63*	-1.06**	-5.66 **	-2.22	-0.51**	-12.66 **	-0.84 **	-5.12**	-2.38 **
TKG-22 X JLT-408	-2.94 **	-2.25 *	-5.29 **	0.37	-0.89**	-0.23	-14.98 **	-0.39 *	-8.75 **	-0.58 **	-4.55**	-2.00 **
TKG-22 X MT-10-8-1	0.23	-1.75	9.17**	3.12*	0.70 **	-0.47	0.08	0.42 *	5.79 **	0.57 **	3.18**	0.62 **
TKG-22 X RT-368	0.52	0.33	8.67 **	5.50**	0.99 **	1.22	9.47 **	0.13	4.57 **	0.54 **	3.85**	0.86 **
TKG-22 X AKT-101	0.85	-0.13	18.59 **	7.73**	0.68 **	3.47 **	-2.64	0.50	-2.91 **	-0.52 **	3.37**	-0.04
IC 204845 X JLT-408	2.73 **	5.80 **	11.81 **	2.78*	-0.14	2.77 *	-6.45 **	-0.36 *	-4.70 **	-0.40 **	-4.44**	-1.13 **
IC 204845 X MT-10-8-1	0.39	1.79	-9.08 **	-1.13	-0.25	1.53	5.61 **	0.40 *	0.74	-0.13	1.42	0.67 **
IC 204845 X RT-368	0.19	-0.13	0.41	-2.20	0.34 *	-0.48	2.00	-0.06	-0.48	-0.52 **	-2.70*	-0.35 **
IC 204845 X AKT-101	-1.48 *	-1.58	16.29 **	2.58*	-0.27	3.97 **	-11.22 **	0.11	5.54 **	0.08	6.49**	0.94 **
JLT-408 X MT-10-8-1	2.97 **	1.75	3.34 *	2.12	-0.18	-0.94	-2.95 *	0.21	-2.85 **	-0.38 **	2.31*	-0.01
JLT-408 X RT-368	-1.73*	-1.17	0.43	1.50	-0.30	-2.35 *	6.74 **	-0.27	-2.85 **	-0.41 **	-2.97*	-1.38 **
JLT-408 X AKT-101	-1.40 *	-3.13 **	-10.49 **	3.13*	0.09	-0.30	-4.18 **	0.09	-3.75 **	0.06	-1.97	-0.85 **
MT-10-8-1 X RT-368	-1.06	2.33 *	-4.86 **	-6.00**	-0.51**	-0.49	-11.30 **	-0.05	-6.93 **	-0.71 **	-6.14**	-1.81 **
MT-10-8-1 X AKT-101	1.27	0.38	0.02	1.68	-0.01	-5.54 **	-6.32 **	-0.01	2.09	-0.18	-2.84*	-0.50 **
RT-368 X AKT-101	0.06	0.96	-0.79	-6.00**	-0.03	-1.55	3.87 **	0.08	0.97	0.14	-1.03	0.31 **

*- Significant at 5% level **-Significant at 1% level

non-additive gene action in hybrids for this trait. Whereas, Musibau and Joseph (2014) and Saxena *et al*. (2017) noticed additive gene action.

For oil content, among the parents DS-5, MT-10-8-1, JTS-8, and TKG-22 were identified as good general combiners based on higher gca effects for this trait. Among the hybrids, IC-204845 x AKT-101 (6.49%) reported highest positive sca effects followed by TKG-22 x RT-368 (3.85%), JTS-8 x RT-368 (3.61%) and DS-5 x IC-204845 (3.60%). The low and non-significant parental gca effects were noticed in the cross JTS-8 x RT-368.

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00011.4

Table 4: Estimates of combining ability variance components and their ratios for twelve characters in sesame

Characters	GCA	SCA	GCA /SCA	Additive variance	Dominance variance	Additive/ Dominance variance
Days to 50% flowering	0.99	5.46	0.18	1.98	5.46	0.36
Days to maturity	0.42	10.92	0.04	0.84	10.92	0.08
Plant height (cm)	4.32	65.02	0.07	8.63	65.02	0.13
Height to first capsule (cm)	1.15	20.58	0.06	2.29	20.58	0.11
Number of branches per plant	0.03	0.25	0.12	0.06	0.25	0.24
Number of capsules on main stem	0.95	13.46	0.07	1.90	13.46	0.14
Number of capsules per plant	15.06	80.67	0.19	30.13	80.67	0.37
Capsule length (cm)	0.01	0.07	0.08	0.01	0.07	0.17
Number of seeds per capsule	3.10	53.63	0.06	6.21	53.63	0.12
1000 seed weight (g)	0.02	0.37	0.08	0.05	0.37	0.15
Oil content (%)	0.82	9.25	0.09	1.64	9.25	0.18
Seed yield per plant (g)	0.13	1.82	0.07	0.26	1.82	0.14

Table 5: *Per se* performance of entries involved in Half-diallel analysis in sesame

Hybrids	Days to 50 per cent flowering	Days to maturity	Plant height (cm)	Height to first capsule (cm)	No. of branches per plant	No. of capsules on main stem	No. of capsules per plant	Capsule length (cm)	No. of seeds capsule^{-1}	1000 seed weight (g)	Oil content (%)	Seed yield plant^{-1} (g)
DS-5 x DSS-9	50.00	100.50	124.00	40.45	4.40	24.00	58.50	2.77	57.60	3.69	48.30	7.95
DS-5 X JTS-8	51.50	108.00	132.20	54.50	4.50	24.60	62.00	3.19	65.30	4.14	49.45	9.00
DS -5X RMT-430	48.50	102.50	122.60	48.60	4.60	24.60	51.00	2.59	48.80	2.46	46.40	5.94
DS-5 X TKG-22	50.50	102.00	129.90	49.50	4.80	26.40	57.50	2.73	55.40	2.57	45.35	7.36
DS-5 X IC 204845	43.00	98.50	123.90	41.00	4.10	32.55	66.00	3.19	63.30	3.60	43.35	8.19
DS-5 X JLT-408	47.50	100.00	135.00	47.20	4.80	25.00	64.00	2.91	61.40	3.00	51.10	8.00
DS-5 X MT-10-8-1	42.00	96.00	130.80	47.00	4.80	32.30	52.50	2.78	56.50	3.74	46.88	7.50
DS-5 X RT-368	42.50	97.50	138.90	51.90	4.70	36.50	64.20	2.70	54.40	2.92	50.28	7.25
DS-5 X AKT-101	49.00	100.00	136.40	39.60	4.50	29.80	75.80	3.40	67.50	4.15	49.48	9.00
DSS-9 X JTS-8	43.00	98.00	136.00	38.30	4.50	26.10	59.60	2.46	49.40	2.57	43.55	5.74
DSS-9 X RMT-430	43.00	95.50	128.00	37.80	4.10	29.60	53.30	2.70	55.40	2.86	46.00	7.41
DSS-9 X TKG-22	44.00	102.00	126.80	47.60	4.50	20.60	62.30	2.76	60.90	3.21	47.80	7.75
DSS-9 X IC 204845	48.00	98.50	123.60	45.40	4.30	29.90	51.50	2.37	48.10	2.47	41.75	4.94
DSS-9 X JLT-408	43.50	96.50	117.80	48.00	4.40	36.00	63.65	2.68	51.20	2.68	46.11	6.68
DSS-9 X MT-10-8-1	43.50	98.00	138.30	53.45	4.30	32.80	81.30	2.76	56.80	3.90	48.71	7.94
DSS-9 X RT-368	41.00	92.00	120.30	45.40	4.30	32.10	62.40	2.70	51.40	2.80	45.70	7.12
DSS-9 X AKT-101	44.50	96.50	118.60	44.10	4.50	23.10	77.20	2.56	50.70	2.62	42.6	6.24
JTS-8 X RMT-430	49.50	106.50	129.20	46.10	4.10	24.40	63.30	2.82	58.70	3.84	48.72	7.95
JTS-8 X TKG-22	49.50	103.00	124.00	43.50	3.30	23.80	60.20	2.59	48.10	2.54	40.60	5.78
JTS-8 X IC 204845	45.50	96.50	121.00	41.30	3.30	28.60	55.80	2.64	51.30	2.80	42.40	6.35
JTS-8 X JLT-408	48.50	101.50	119.70	36.30	3.30	37.80	63.40	2.85	53.60	3.11	47.62	7.40
JTS-8 X MT-10-8-1	44.50	99.00	121.90	42.50	3.90	30.00	65.70	3.15	73.30	4.11	47.35	9.73
JTS-8 X RT-368	49.50	98.00	126.00	50.90	4.50	34.20	67.40	2.53	49.80	2.69	47.00	6.00
JTS-8 X AKT-101	50.00	99.00	138.50	50.50	4.80	23.90	69.10	3.29	74.00	4.27	49.34	11.00
RMT-430 X TKG-22	46.50	99.50	123.75	44.40	3.70	29.90	49.80	3.00	63.40	3.90	46.51	8.30
RMT-430 X IC 204845	47.00	95.00	126.90	48.20	3.50	24.70	51.60	2.60	54.10	2.56	49.75	6.11
RMT-430 X JLT-408	49.00	102.00	122.80	44.00	3.30	31.00	42.40	2.66	58.70	2.80	44.50	6.56
RMT-430 X MT-10-8-1	51.00	108.00	139.30	50.55	4.50	26.20	74.20	3.10	72.50	4.20	48.95	9.23
RMT-430 X RT-368	47.00	97.50	110.30	33.80	3.10	29.90	45.60	2.43	45.70	2.68	40.90	5.33
RMT-430 X AKT-101	48.00	95.50	130.30	51.40	5.10	30.90	90.10	3.16	67.10	4.15	41.40	8.58
TKG-22 X IC 204845	49.50	102.50	114.10	42.80	3.50	25.70	61.08	3.00	59.00	3.23	48.90	8.00
TKG-22 X JLT-408	50.50	97.50	116.00	45.00	3.20	32.30	53.40	3.18	64.40	4.00	47.93	8.55
TKG-22 X MT-10-8-1	49.00	98.00	126.90	50.20	4.00	30.00	64.50	2.88	59.70	3.21	48.00	7.66

Table 5: Contd....

Hybrids	Days to 50 per cent flowering	Days to maturity	Plant height (cm)	Height to first capsule (cm)	No. of branches per plant	No. of capsules on main stem	No. of capsules per plant	Capsule length (cm)	No. of seeds capsule^{-1}	1000 seed weight (g)	Oil content (%)	Seed yield plant^{-1} (g)
TKG-22 X RT-368	50.00	100.00	114.60	43.70	4.30	32.00	68.00	2.64	49.60	2.76	43.75	6.00
TKG-22 X AKT-101	47.50	101.00	121.60	46.30	4.70	28.60	74.55	3.26	69.10	4.14	50.40	10.24
IC 204845 X JLT-408	48.00	97.50	110.60	46.20	3.10	25.50	68.20	2.38	45.80	2.44	41.20	5.24
IC 204845 X MT-10-8-1	43.00	97.00	118.50	46.20	3.30	28.80	46.50	2.50	46.90	2.52	41.40	5.53
IC 204845 X RT-368	46.50	97.50	130.00	49.80	4.70	30.30	61.00	3.10	64.60	3.95	50.10	8.27
IC 204845 X AKT-101	47.50	99.50	133.80	51.30	5.00	30.10	71.30	3.00	63.50	3.85	49.75	8.19
JLT-408 X MT-10-8-1	47.00	99.00	141.80	53.20	4.70	34.40	52.90	2.88	53.10	2.46	47.22	6.82
JLT-408 X RT-368	48.00	102.00	116.80	47.60	3.80	26.70	69.90	2.89	59.30	3.45	47.23	7.76
JLT-408 X AKT-101	50.50	106.50	139.90	51.80	4.00	31.10	61.40	2.42	49.00	2.49	41.40	5.93
MT-10-8-1 X RT-368	48.50	102.50	116.00	48.75	3.70	31.60	72.90	2.96	57.60	3.05	48.23	7.86
MT-10-8-1 X AKT-101	49.00	100.50	129.80	46.80	4.30	27.70	70.20	2.74	56.50	2.59	43.10	6.51
RT-368 X AKT-101	46.50	99.00	143.80	51.25	3.70	34.20	50.70	2.82	59.60	2.86	50.23	7.34
Mean	48.14	101.29	131.7	46.38	4.14	29.12	62.40	2.82	57.38	3.20	46.37	7.38
CD @ 5%	**1.37**	**2.2**	**3.04**	**2.55**	**0.31**	**2.21**	**2.71**	**0.32**	**2.10**	**0.11**	**2.30**	**0.16**
CD @ 1%	**1.83**	**2.95**	**4.06**	**3.40**	**0.42**	**2.96**	**3.62**	**0.42**	**2.80**	**0.15**	**3.06**	**0.22**
Parents												
DS-5 (SC)	45.50	97.00	129.30	39.00	4.60	24.20	67.40	2.88	53.60	3.10	48.09	7.41
DSS-9	45.50	93.00	127.60	49.00	3.80	27.00	55.40	2.79	51.20	2.62	48.75	7.00
JTS-8 (ZC)	41.50	95.00	129.00	47.50	3.70	23.70	66.00	2.54	45.80	2.51	42.45	5.40
RMT-430	41.00	93.00	116.20	48.80	4.10	27.08	48.80	2.80	47.50	2.65	41.40	5.46
TKG-22 (NC)	44.00	94.50	121.10	47.60	3.90	29.70	62.30	2.63	56.60	3.52	48.95	7.38
IC 204845	42.50	95.50	120.70	40.90	3.30	27.30	47.40	2.55	50.40	2.50	40.25	5.00
JLT-408	44.00	96.50	123.70	48.20	3.80	24.30	46.10	2.50	56.50	2.70	41.50	5.94
MT-10-8-1	44.50	96.00	124.60	48.20	3.60	27.70	47.80	2.73	58.10	3.19	44.00	6.54
RT-368	43.50	92.00	127.20	39.75	3.80	26.40	57.20	2.82	55.50	2.95	42.30	6.42
AKT-101	42.00	96.00	118.20	46.00	4.00	31.80	50.60	2.82	57.40	3.12	41.10	6.85
Mean	43.4	94.85	123.76	45.50	3.86	26.91	54.90	2.71	53.26	2.89	43.88	6.34
CD @ 5%	**0.46**	**0.74**	**1.01**	**0.85**	**0.10**	**0.74**	**0.90**	**0.11**	**0.70**	**0.04**	**0.77**	**0.06**
CD @ 1%	**0.65**	**1.06**	**1.46**	**1.22**	**0.15**	**1.06**	**1.30**	**0.15**	**1.00**	**0.05**	**1.10**	**0.08**
CV (%)	**5.77**	**3.70**	**6.45**	**10.25**	**13.66**	**13.86**	**16.16**	**9.20**	**12.65**	**18.85**	**7.57**	**18.19**

SC- Standard check ZC- Zonal check NC- National check

Apart from the seed yield, the improvement of oil content is an important goal of the breeders. Based on combining ability variance for this trait, it revealed that SCA variance was more than GCA variance and that non-additive gene action predominated. Therefore it is possible to increase the oil content by heterosis breeding. Related findings suggesting non-additive gene action were observed for this trait by Musibau and Joseph (2014); Joshi *et al*. (2015) and Meenakumari *et al*. (2015). Whereas on contrary Parameshwarappa and Salimath (2010); Vekaria *et al*. (2014) and Balaram *et al*. (2018) reported additive gene action for this trait.

For seed yield per plant, gca effects of parents suggested that TKG-22, DS-5, RMT-430 and JTS-8 were the good general combiners. Among twenty-one, eighteen hybrids were showed significant positive sca effects and the highest sca effects observed in JTS-8 x JLT-408 (3.73 per cent) followed by JTS-8 x TKG-22 (1.90 per cent), DS-5 x RT-368 (1.76 per cent) and DS-5 x DSS-9 (1.51 per cent) and showed positive heterosis and significant gca effects of parents involved in the all these hybrids. These results in general indicated the possible relationship among the sca effect, heterosis and the observed *per se* performance of the hybrids for this trait (Table 3).

Improvement of seed yield is the primary objective of any plant breeding programme. SCA variance for this trait was more than the GCA variance suggesting the role of non-additive gene action. Thus, it is proposed that the yield may be improved by exploiting heterosis. Related results suggesting non-additive gene function were also reported by Vidhyavathi *et al*. (2005). Similar findings of involvement of non-additive gene action was also found by Musibau and

doi: 10.5958/2582-2683.2023.00011.4

Joseph (2014); Meenakumari *et al*. (2015) and Balaram *et al*. (2018). Whereas, Saxena *et al*. (2017) reported the involvement of additive gene action.

Based on *per se* performance, sca effects and gene action involved in the expression of seed yield and its components, the hybrids JTS-8 X MT-10-8-1 (9.73 g), RMT-430 X MT-10-8-1 (9.23 g), IC-204845 X AKT-101 (8.19 g), DSS-9 x RT-368 (7.12 g) and RMT-430 x IC-204845 (6.11 g) were considered as best hybrids for exploitation of heterosis for seed yield. The hybrids such as TKG-22 X AKT-101 (50.4%), DS-5 X RT-368 (50.28%), IC-204845 X AKT-101 (49.75%), TKG-22 x RT-368 (43.75%) and DSS-9 x JTS-8 (43.55%) can be best exploited for oil content (Table 5). Superior crosses should be studied for isolating superior plant types in F_2 and subsequent generations. DS-5 should be involved in crossing programme and the generated F_1's could be utilized in future breeding program to release early maturing, good quality and high yielding varieties which will ultimately increase the productivity and decrease the gap between demand and domestic production of edible oil.

CONCLUSION

The study concludes that the standard check DS-5 and RMT 430 are the best general combiner for most of the traits followed by IC-204845. JTS-8 X MT-10-8-1, RMT-430 X MT-10-8-1 and IC-204845 X AKT-101 were identified to be top promising heterotic hybrids for seed yield over standard check DS-5. The hybrids TKG-22 X AKT-101, DS-5 X RT-368, IC-204845 X AKT-101, TKG-22 x RT-368 and DSS-9 x JTS-8 can be best exploited for oil content in Sesame. The results suggest that heterosis coupled with high sca effects may be considered as a criterion for selecting the best cross combination for further improvement of seed yield and its attributes should be utilized in multiple crossing programmes in Sesame.

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Online Manuscript No. 97926 recived on 1.5.2022
Manuscript accepted for publication on 14.11.2022

Journal of Eco-friendly Agriculture **18**(1): 68-71. 2023
doi: 10.5958/2582-2683.2023.00012.6

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

A study of character association and genetic divergence in bread wheat (*Triticum aestivum* L.)

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ABSTRACT

An experiment with 50 genotypes of wheat (*Triticum aestivum* L.) was conducted to study the character association and genetic divergence on various quantitative characters with grain yield per plant. The maximum and positive phenotypic correlation coefficient was observed for seeds per plant with biological yield per plant, harvest, index, 100-grain weight, and the number of grains per ear. The path coefficient analysis at the phenotypic level revealed that harvest index, biological yield per plant, and days of 50 per cent flowering showed a positive direct effect on grain yield per plant. Hence, selection based n these characters would bring an improvement in *T. aestivum*. The genotypes were grouped into eight clusters. Cluster VII was the biggest with 11 genotypes followed by clusters II, IV, I, and V with nine, eight, six, and six, respectively. The maximum inter-cluster distance observed between clusters VIII and III was 4.995 which showed maximum genetic diversity between these two clusters followed by clusters VII and VI (4.648), clusters VII and II (4.644), clusters III and I (4.641) and clusters VIII and VII (4.318) indicating wide divergence among these clusters, which also suggested that the genetic architecture of the genotypes in one cluster differed entirely from those included clusters. The diversity among the genotypes measured by inter-cluster distance (D value) was adequate for *T. aestivum* by hybridization and selection.

***Key words*:** Genetic divergence, correlation, *Triticum*, path coefficient

Wheat (*Triticum aestivum* L. em. Thell) is the second most important staple food crop of the world after rice. However, it is cultivated widely around the world due to wide adaptation and a greater role in human nutrition as well as in the agricultural economy. Yield is a complex character and is the product of a number of characters, each of which is under polygenic control. The correlation coefficient a statistical tool is used to find out the degree and direction of the relationship between two or more variables. In plant breeding, correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters and thus becomes an integral component for selection aimed at yield improvement. The coefficient analysis elucidates the intrinsic nature of the observed association between yield and its attributes. The most simple and convenient methods for working out the genetic diversity among the genotypes are available in the form of D^2 analysis for grouping or clustering of genotypes/parents. Therefore, the present investigation was undertaken to gather information on these aspects of wheat (*T. aestivum*).

MATERIALS AND METHODS

The present investigation and experiment were carried out in the Janta Vedic College Baraut, Baghpat, Uttar Pradesh, India. The 50 genotypes were sown in a Randomized Block Design with three replications and three rows per genotype in each replication. Five competitive plants from each plot were randomly selected from all three rows for recording data. Observation of 10 characters *viz*; Days to 50 per cent flowering, Days to 80 per cent maturity, Number of tillers per plant, Plant height (cm), Ear length (cm), Grains per ear, 100-grain weight (g), Grain yield per plant (g), Biological yield per plant (g), Harvest index (%) was recorded on single plant basis except for days to flowering and days to maturity. The average of these selected five plants in respect of different plant characters was used for statistical analysis. Phenotypic correlations were estimated as per Panse and Sckhatme (1967). The path coefficient was estimated as suggested by Dewey and Lu (1959) and the genetic divergence was estimated as suggested by Mahalanobis (1936).

RESULTS AND DISCUSSION

Adequate knowledge about the nature and magnitude of the association of characters is a prerequisite for operating an efficient selection programme. Exhaustive studies on the interrelationship of characters among them and also between yield and its component traits have been carried out in the past in various crops. The genotypic and phenotypic correlation coefficients among 10 characters in 50 genotypes of wheat were calculated. The results are presented in Table 1. At the phenotypic level, the Harvest index showed a highly

significant positive correlation with grain yield per plant (0.823) and the number of grains per ear (0.428), 100-grain weight (0.374), and biological yield (0.208). It showed a negative but non-significant correlation with days to 50 per cent flowering (-0.151), days to 80% maturity (-0.234), plant height (-0.135), and ear length (-0.090). It had a positive but non-significant correlation with the number of tillers per plant (0.110). Grain yield per plant showed a highly significant positive correlation with harvest index (0.823), biological yield (0.721), 100-grain weight (0.529), and the number of grains per ear (0.456) while, it showed a negative correlation with days to 50 per cent flowering (-0.311). Grain yield per plant had a negative but non-significant correlation with days to 50 per cent flowering (-0.156), the number of tillers per plant (-0.060), and ear length (-0.234). Biological yield per plant showed a highly significant positive correlation with 100-seed weight (0.462), number of grains per ear (0.302), and plant height (0.282). 100-grain weight showed a positive but non-significant correlation with days of 50 per cent flowering (0.097), the number of tillers per plant (0.060), and plant height (0.091). The number of grains per ear showed a significant positive correlation with ear length (0.302). Similar results were obtained by Uddin *et al*. (1997), Sachin and Singh (2003), Lad *et al.* (2003), Prasad *et al.* (2006), Singh *et al.* (2009), Devesh *et al.* (2021) and Rathod *et al.* (2019).

The path coefficient analysis was used to find out the direct and indirect effect of the nine different characters towards grain yield per plant. The estimates of direct and indirect effects are presented in Table 2. It may be noted that the path analysis, positive direct effect on grain yield per plant was exerted by biological yield per plant, harvest index, and days to 50 per cent flowering. The negative direct effect on grain yield per plant was exerted by days to maturity, number of tillers per plant, plant height, ear length, number of grains per ear, and 100-grain weight.

To bring out the improvement in yield, direct selection for seed yield per plant has been the most common method used by plant breeding in self-pollinated crops. However, it is possible to achieve the selection efficiency for yield even by indirect selection especially if secondary characters are

Table 1. Phenotypic correlation coefficient for ten quantitative characters in wheat genotypes

Characters	Days to 50% flowering	Days to 80% maturity	No. of tillers per plant	Plant height (cm)	Ear length (cm)	No. of grain per ear	100-grain weight (g)	Biological yield/plant	Grain yield/ plant	Harvest index (%)
Days to 50% flowering	1.000	0.800**	-0.103	-0.335**	-0.028	-0.131	0.097	-0.119	-0.156	-0.151
Days to 80% maturity		1.000	-0.105	-0.294	0.077	-0.148	-0.036	-0.263*	-0.311**	-0.234
No. of tillers per plant			1.000	0.010	0.081	-0.118	0.060	-0.234	-0.060	0.110
Plant height (cm)				1.000	-0.194	0.016	0.091	0.282*	0.058	-0.135
Ear length (cm)					1.000	0.302*	-0.397**	-0.275*	-0.234	-0.090
No. of grain per ear						1.000	-0.111	0.302*	0.456**	0.428**
100-grain weight (g)							1.000	0.462**	0.529**	0.374
Biological yield/plant								1.000	0.721**	0.208
Grain yield/plant									1.000	0.823**
Harvest index (%)										1.000

*Significant at 5%, **Significant at 1%

Table 2. Phenotypic direct and indirect effects for different quantitative characters in wheat genotypes

Characters	Days to 50% flowering	Days to 80% maturity	No. of tillers per plant	Plant height (cm)	Ear length (cm)	No. of grain per ear	100-grain weight (g)	Biological yield/plant	Harvest index (%)	'r' with Grain yield/ plant
Days to 50% flowering	**0.0410**	-0.0210	0.0000	0.0010	0.0000	0.0040	-0.0020	-0.0700	-0.1090	-0.156
Days to 80% maturity	0.0330	**-0.0260**	0.0000	0.0010	0.0000	0.0040	0.0010	-0.1550	-0.1690	-0.311*
No. of tillers per plant	-0.0040	0.0030	**-0.0020**	0.0000	0.0000	0.0040	-0.0010	-0.1370	0.0800	-0.060
Plant height (cm)	-0.0140	0.0080	0.0000	**-0.0030**	0.0010	0.0000	-0.0020	0.1650	-0.0970	0.058
Ear length (cm)	-0.0010	-0.0020	0.0000	0.0010	**-0.0050**	-0.0090	0.0090	-0.1610	-0.0650	-0.234
No. of grain per ear	-0.0050	0.0040	0.0000	0.0000	-0.0010	**-0.0300**	0.0020	0.1770	0.3090	0.456**
100-grain weight (g)	0.0040	0.0010	0.0000	0.0000	0.0020	0.0030	**-0.0230**	0.2720	0.2700	0.529**
Biological yield/plant	-0.0050	0.0070	0.0010	-0.0010	0.0010	-0.0090	-0.0100	**0.5870**	0.1500	0.721**
Harvest index (%)	-0.0060	0.0060	0.0000	0.0000	0.0000	-0.0130	-0.0080	0.1220	**0.7220**	0.823**

highly correlated with yield and is easily measurable, Earlier studies on path analysis in wheat conducted by Dhonde (2000), Singh and Singh (2001), Ayer *et al.* (2017) and Poudel *et al.* (2021) also reported similar results. The knowledge may be successfully utilized for selecting parents for hybridization, divergence analysis is performed to identify the diverse genotype for hybridization purposes because of that the greater diversity among the genotypes had more chances for producing superior heterotic combinations and transgressive segregated among the progenies of diverse crosses. In the present study, all 50 genotypes were grouped into eight clusters by employing Mahalanobis D^2 techniques. Table 3 presents the pattern of distribution of these genotypes into various clusters which showed considerable genetic diversity among the genotypes for most of the traits studied. The role of different plant traits in the inter-cluster divergence can further be studied by comparing cluster mean for different characters. Intra and inter-cluster distances of 50 genotypes are presented in Table 4 and the cluster means for different characters are depicted in Table 5.

The highest intra-cluster distance was observed in cluster VIII indicating that genotypes in this cluster were genetically more divergent. The inter-cluster distance between clusters VIII and III was observed highest (4.995) and the lowest inter-cluster value was observed between clusters III and IV (2.337). Cluster IV had 11 genotypes with an intra-cluster distance of 2.414. Cluster II had 9 genotypes with an intra-cluster distance of 2.164 and cluster IV had 8 genotypes with an intra-cluster distance of 1.786. Clusters I and VI had 6 genotypes with intra-cluster distances of 1.823 and 1.786, respectively. Cluster VIII has 4 genotypes with an intra-cluster distance of 2.539. Clusters III and VI had 3 genotypes each with inter-cluster distances of 1.804 and

Table 3. Distribution of 50 genotypes in *Triticum aestivum* in eight clusters

Characters	No of genotypes	Name of Genotypes
I	6	HS-295, TL-2942, VL-892, VL-907, HI-1563, NW-4035
II	9	DBW-50, PBW-621, PBW-315, PBW-317, PDW-291, WH-896, HD-1997, RSP-561, HD-2967
III	3	HS-0513, HPW-215, HS-240
IV	8	C-306, PBW-175, PBW-373, PBW-395, DBW-51, DBW-52, HD-3016, DBW-14
V	6	Sonalika, UAS-35, HD-2967, PBW-590, PDW-233, DBW-39
VI	3	VL-925, WH-1080, WH-1081
VII	11	HS-507, HS-277, HS-375, HS-490, VL-616, VL-804, VL-829, PBW-629, UP-2744, PBW-550, DBW-46
VIII	4	WHD-943, MACS-2496, PDW-314, WH-1021

Table 4. Estimation of average intra and inter-cluster distance or what for eight clusters constructed for 50 genotypes in Wheat

Characters	I	II	III	IV	V	VI	VII	VIII
I	**1.823**							
II	3.252	**2.164**						
III	4.641	4.406	**1.804**					
IV	3.402	2.791	2.624	**1.786**				
V	2.451	3.531	3.593	2.971	**1.932**			
VI	3.361	3.620	3.588	3.669	2.843	**1.412**		
VII	3.950	4.644	2.970	2.337	3.217	4.648	**2.414**	
VIII	3.593	3.669	4.995	3.469	4.321	3.955	4.318	**2.539**

Table 5. Mean values of 10 characters in eight clusters constructed from 25 genotypes of wheat

Characters	Days to 50% flowering	Days to 80% maturity	No. of tillers per plant	Plant height (cm)	Ear length (cm)	No. of grain per ear	100-grain weight (g)	Biological yield/plant	Grain yield/ plant	Harvest index (%)
I	68.50	86.58	4.53	88.62	8.98	52.53	3.10	15.31	8.04	52.56
II	73.78	89.39	4.08	70.80	8.96	53.58	3.61	16.16	10.36	64.19
III	79.83	96.50	5.58	64.63	10.61	56.67	2.71	12.63	7.37	58.58
IV	78.19	96.25	4.24	72.62	10.12	49.10	3.36	13.95	8.05	57.82
V	67.67	86.50	4.62	73.08	9.99	45.99	2.79	13.28	7.50	56.57
VI	70.67	86.33	6.32	75.17	10.32	49.07	3.44	13.94	8.78	62.97
VII	78.50	96.36	4.38	71.21	9.68	44.09	2.77	13.36	6.07	45.49
VIII	76.25	93.25	5.12	83.99	5.79	40.20	4.19	15.20	8.45	55.61

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00012.6

1.412, respectively. The relationship between genetic and geographical diversity has been a point of debate in the past. But no definite conclusion regarding the relationship between the genetic and geographical diversity of the strains is not as much influenced by one geographical distribution but by their pedigrees (Kant *et al*., 1999; Singh and Dwivedi, 2002; Kumar *et al*., 2018).

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Online Manuscript No. 99326 recived on 31.7.2022
Manuscript accepted for publication on 18.11.2022

Journal of Eco-friendly Agriculture **18**(1): 72-79. 2023
doi: 10.5958/2582-2683.2023.00013.8

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Elucidation of GxE interactions by AMMI, BLUP and non-parametric measures of Wheat genotypes evaluated in NWPZ

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ABSTRACT

Significant variations due to environments (38.3%), GxE interactions (32.1%), and genotypes (10.9%) were observed by AMMI analysis. Absolute IPCA-1 scores pointed for G13, G2, G11 as per IPCA-2, G1, G3, G8 genotypes would be of choice. ASV1 and ASV measures recommended G13, G2, G11 genotypes. Based on 97.8 per cent of GxE interactions sum of squares MASV1 identified G13, G3, G7 whereas MASV settled for G13, G1, and G7 genotypes. BLUP-based measures HMGV, RPGV, HMRPGV identified G9, G6, G13 genotypes for this mega wheat producing zone of the country. Non parametric composite measure $NP_i^{(1)}$ observed suitability of G3, G13, G12 whereas $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$ identified G3, G10, G12 genotypes. Biplot analysis observed 65.4% of the total variation in the considered measures accounted by PC1 and PC2 with respective contributions of 35.4% & 29.9% respectively. S_i^1, S_i^2, S_i^4, S_i^3, $NP_i^{(1)}$, S_i^6, ASV, ASV1, IPC7 accounted more in PC1 whereas for second component RPGV HMRPGV, HMGV, BLAvg, BLGM, $NP_i^{(4)}$ were major contributors. Cluster of IPC2, $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$ placed adjacent to BLUP based measures in the same quadrant. ASV, ASV1, MASV, MASV1, clustered with S_i^1, S_i^2, S_i^3, S_i^4, S_i^5, S_i^6, S_i^7 BLStd and $NP_i^{(1)}$, BLCV, IPC7 observed in different quadrant of biplot analysis. Strong positive Spearmen rank correlations of ASV & ASV1 seen of moderate to strong direct nature with measures exception of $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$. MASV, MASV1 expressed moderate to strong positive correlation values S_i^1, S_i^2, S_i^3, S_i^4, S_i^5, S_i^6, S_i^7, $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$. Similar type of relationships were expressed by BLUP based measures.

Keywords: AMMI, BLUP, Biplot analysis, Non parametric composite measures

Multi-environment trials have been advocated for the selection the better performing stable yield across various environments before the wide scale cultivation. Precise and valuable information about cultivar performance in different environments have been provided by genotype × environment interaction effects. A use of recent analytic approaches in analyzing the genotype × environment has been advocated in recent studies to (Pour Aboughadareh *et al.*, 2022). The consideration of accumulation of main effects of genotypes, environments with their multiplicative interactions have been used in Additive main effect and multiplicative interaction (AMMI) model in latest studies as compared to joint regression analysis (Pour-Aboughadareh *et al.*, 2019). AMMI based measures (AMMI stability value (ASV), ASV1, Modified AMMI stability value (MASV) & MASV1) have also gained visibility (Sousa *et al.*, 2020). Best linear unbiased prediction (BLUP) based measures offers the potential to improve the predictive accuracy of random effects, harmonic mean of genotypic values (HMGV), relative performance of genotypic values (RPGV), and harmonic mean of relative performance of genotypic values (HMRPGV), were also highlighted for the stability and adaptability of genotypes (Gonçalves *et al.*, 2020). Besides that nonparametric measures S_i^1 S_i^2 S_i^3 S_i^4 S_i^5 S_i^6 S_i^7, $NP_i^{(1)}$, $NP_i^{(2)}$, $NP^{(3)}$, $NP_i^{(4)}$ have been also utilized to interpret the response of genotypes to environmental conditions (Pour-Aboughadareh *et al.*, 2019). Recent analytic measures have been compared to decipher the GxE interactions effects for wheat genotypes evaluated in north western plains zone of the country under timely sown irrigated conditions.

MATERIALS AND METHODS

Fourteen promising wheat genotypes were evaluated in research field trials at 14 centers of All India Coordinated Research Project on wheat across this zone of the country during 2020-21 cropping season in field trials for restricted irrigation conditions. More emphasis had been placed to increase the wheat production of this zone to augment the total cereal production of the country. Field trials were laid out in Randomized block designs with four replications. Recommended practices of packages had followed in total to harvest the good yield. Parentage details and environmental conditions were reflected in table 1 for ready reference. Pour-Aboughadareh *et al.*, 2019 recommended various non parametric and parametric measures for assessing GxE interaction and stability analysis. For a two-way dataset with k genotypes and n environments X_{ij} de-notes the phenotypic value of ith genotype in jth

environ-ment where *i*=1,2, ...*k*, ,*j* =, 1,2 ,...,*n* and r_{ij} as the rank of the ith genotype in the jth environment, and $\overline{r_i}$ as the mean rank across all environments for the ith geno-type. The correction for yield of ith genotype in jth environment as ($X^*_{ij} = X_{ij} - \overline{x_i}. + \overline{x_{..}}$) as X^*_{ij}, was the corrected phenotypic value; $\overline{X_i}.$ was the mean of ith genotype in all environments and $\overline{X_{..}}$ was the grand mean.

$$S_i^{(1)} = \frac{2\sum_{j=1}^{n-1}\sum_{j'=j+1}^{n}|r_{ij} - r_{ij'}|}{[n(n-1)]} \qquad S_i^{(7)} = \frac{\sum_{j=1}^{n}(r_{ij} - \bar{r}_i)^2}{\sum_{j=1}^{n}|r_{ij} - \bar{r}_i|} \qquad S_i^{(3)} = \frac{\sum_{j=1}^{n}(r_{ij} - \bar{r}_i)^2}{\bar{r}_{i.}}$$

$$S_i^{(4)} = \sqrt{\frac{\sum_{j=1}^{n}(r_{ij} - \bar{r}_i)^2}{n}} \qquad S_i^{(5)} = \frac{\sum_{j=1}^{n}|r_{ij} - \bar{r}_i|}{n} \qquad S_i^{(6)} = \frac{\sum_{j=1}^{n}|r_{ij} - \bar{r}_i|}{\bar{r}_{i.}}$$

$$S_i^{(2)} = \frac{\sum_{j=1}^{n}(r_{ij} - \bar{r}_{i'})^2}{(n-1)} \qquad \bar{r}_i = \frac{1}{n}\sum_{j=1}^{n} r_{ij.}$$

Non parametric composite measures $NP_i^{(1)}$, $NP_i^{(2)}$, $NP_i^{(3)}$ and $NP_i^{(4)}$ based on the ranks of genotypes as per yield and corrected yield of genotypes. In the formulas, r^*_{ij} was the rank of X^*_{ij}, and $\overline{r_i}$ and M_{di} were the mean and median ranks for original (unadjusted) grain yield, where $\overline{r_i}^*$ and M^*_{di} were the same parameters computed from the corrected (adjusted) data.

$$NP_i^{(1)} = \frac{1}{n}\sum_{j=1}^{n} | r_{ij}^* - M_{di}^* | \qquad NP_i^{(3)} = \frac{\sqrt{\Sigma(r_{ij}^* - \bar{r}_{i.}^*)^2/n}}{\bar{r}_{i.}}$$

$$NP_i^{(2)} = \frac{1}{n}\left(\frac{\sum_{j=1}^{n}| r_{ij}^* - M_{di}^* |}{M_{di}}\right) \qquad NP_i^{(4)} = \frac{2}{n(n-1)}\left[\sum_{j=1}^{n-1}\sum_{j'=j+1}^{m}\frac{|r_{ij}^* - r_{ij'}^*|}{\bar{r}_{i.}}\right]$$

AMMISOFT version 1.0 software utilized for AMMI analysis of data sets and SAS software version 9.3 for further analysis

ASV	$ASV = [(\frac{SSIPC\ 1}{SSIPC\ 2}PCI)^2 + (PC2)^2]^{1/2}$
ASV1	$ASV1 = [\frac{SSIPC\ 1}{SSIPC\ 2}(PCI)^2 + (PC2)^2]^{1/2}$
Modified AMMI stability Value	$MASV = \sqrt{\sum_{n=1}^{N-1}\frac{SSIPC_n}{SSIPC_{n+1}}(PC_n)^2 + (PC_{n+1})^2}$
MASV1	$MASV1 = \sqrt{\sum_{n=1}^{N-1}(\frac{SSIPC_n}{SSIPC_{n+1}}PC_n)^2 + (PC_{n+1})^2}$
$HMGV_i$	= Number of environments / $\sum_{j=1}^{k}\frac{1}{GV_{ij}}$ GV_{ij} genetic value of ith genotype in jth environments
Relative performance of genotypic values across environments	$RPGV_{ij} = \sum GV_{ij} / \sum GV_j$
Harmonic mean of Relative performance of genotypic values	$HMRPGV_i$ = Number of environments / $\sum_{j=1}^{k}\frac{1}{RPGV_{ij}}$
Geometric Adaptability Index	$GAI = \sqrt[n]{\prod_{k=1}^{n}\bar{X}_k}$

RESULTS AND DISCUSSION

AMMI analysis

Highly significant variations due to environments, GxE interactions, and genotypes were observed by AMMI analysis (Table 2). This analysis also revealed about 38.3% of the total sum square of variation for yield was due to environments followed by GxE interactions, 32.1 per cent whereas genotypes accounted only 10.9 per cent. Diversity of the testing sites were approved by AMMI analysis (Mehraban *et al.*, 2019). Seven interaction principal components accounted for more than 92.9 per cent interactions sum of square variations. AMMI1 explained a total variation of 39.8 per cent, followed by 14.1 per cent for AMMI2, 12.5 per cent for AMMI3, 8.8 per cent for AMMI4, AMMI5 contributed 7.7 per cent followed by 6.3 per cent and 3.6 per cent by AMMI6, AMMI7, respectively. The first two AMMI components in total showed 53.8 per cent of the total variation indicating the two AMMI components well fit and confirm the use of AMMI model (Pour Aboughadareh *et al.*, 2022). Estimated sums of squares for G×E signal and noise were 87.45 per cent and 12.55 per cent of total G×E, respectively. Early IPCs selectively capture signal, and late ones noise. Accordingly, this much signal suggests AMMI6 or maybe AMMI7. Note that the sum of squares for GxE-signal is 2.56 times that for genotypes main effects. Hence, narrow adaptations are important for this dataset (Vaezi *et al.* 2018). Even just IPC1 alone is 1.16 times the genotypes main effects. Also note that GxE-noise is 0.37 times the genotypes effects. Discarding noise improves accuracy, increases repeatability, simplifies conclusions, and accelerates progress.

Ranking of genotypes as per measures

Since the genotypes yield expressed highly significant variations, mean yield was considered as an important measure to assess the yield potential of genotypes. Mean yield of genotypes selected G9, G6, G13 with lowest yield of G8 (Table 3). This measure is simple, but not fully exploiting all information contained in the dataset. Values of IPCA's in the AMMI analysis indicate stability or adaptability of genotypes. The, greater the IPCA scores reflect the specific adaptation of genotype to certain locations. While, the values approximate to zero were recommended for in general adaptations of the genotype. Absolute IPCA-1 scores pointed for G13, G2, G11 as per IPCA-2, G1, G3, G8 genotypes would be of choice. Values of IPCA-3 favored G3, G5, G12 genotypes. As per IPCA-4, G1, G10, G 13 genotypes would be of stable performance. Genotypes G10, G13 G4 selected as per IPCA5 while values of IPCA6 pointed for G7, G8, G12 and finally IPCA7 observed suitability of G9, G13, G2. First two IPCAs in ASV & ASV1 measures utilized 53.9 per cent of G×E

interaction sum of squares. The two IPCAs have different values and meanings and the ASV and ASV1 parameters using the Pythagoras theorem and to get estimated values between IPCA1 and IPCA2 scores to produce a balanced measure between the two IPCA scores. Also, ASV parameter of this investigation used advantages of cross validation due to computation from first two IPCAs (Silva *et al.*, 2019). Using first two IPCAs in stability analysis could benefits dynamic concept of stability in identification of the stable high yielder genotypes. ASV1 measures recommended (G13, G2, G11) and ASV pointed towards (G13, G2, G3) as of stable performance. Adaptability measures MASV and MASV1 considered all seven significant IPCAs of the AMMI analysis using 97.8 per cent of GxE interactions sum of squares (Gerrano *et al.*, 2020). Values of MASV1 identified G13, G3, G7 genotypes would express stable yield whereas genotypes G13, G1, G7 be of stable yield performance by MASV measure, respectively. Major advantages of BLUP based measures are to account for the random nature of the genotype behavior in changes climatic conditions. At the same time allow ranking genotypes in relation to their performance based on the genetic effects (Sousa *et al.*, 2020). Average yield of genotypes

Table 1: Parentage and location details under multi environmental trials of wheat genotypes

Genotype	Code	Parentage	Code	Locations	Latitude	Longitude	Altitude
WH1105	G 1	MILAN/S87230//BABAX	E 1	Delhi	28 °4'N	77 °13 'E	228
DBW187	G 2	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU	E 2	Jammu	32 ° 40' N	74 ° 54'E	356
HD3349	G 3	HD2932/HD3086	E 3	Ludhiana	30 ° 54' N	75 ° 48 'E	247
PBW876B	G 4	SHAKTI/6/KAUZ//ALTAR84/AOS/3/PASTOR/4/873.97/5/MUNAL#1/7/F RET2*2/ SHAMA//KIRITATI/2*TRCH/3/BAJ#1	E 4	Gurdaspur	30° 02' N	75 ° 24 'E	265
HD3406M	G 5	HD2967*3/Trinakriya(LrTrk/YrTrk)	E 5	Rauni	30 ° 90'N	75 ° 85'E	262
DBW222	G 6	KACHU/SAUAL/8/ATTILA*2/PBW65/6/PVN//CAR422/ANA/5/BOW/CROW// BUC/PVN/3/YR/4/TRAP#1/7/ATTILA/2*PASTOR	E 6	Faridkot	30 ° 40 'N	74 ° 04 'E	200
DBW313#	G 7	REH/HARE/2*/BCN/3/CROC/AE.SQUAROSA(213)//PGO/4/HUITE S/ 5/PBW585//PBW509/PBW581	E 7	Hisar	29 ° 10' N	75 ° 46'E	229
HD2967	G 8	ALD/CUC//URES/HD2160M/HD2278	E 8	Karnal	29 ° 43' N	70 ° 58'E	245
PBW826	G 9	WBLL1*2/KKTS//PASTOR/KUKUNA/3/KINGBIRD#1//INQALAB91*2/ TU KURU/5/KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/SAUAL	E 9	Rohtak	28 ° 53' N	76 ° 35'E	222.5
RAJ4548#	G 10	GLADIUS/5/2*W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	E 10	Shikohpur	28 ° 36' N	76 °98'E	259
HD3354	G 11	QUAIU#1/3/T.DICOCCONPI94625/AE.SQUARROSA(372)//3*PASTOR/ 4/QUAIU#2/5/VALI/6/BECARD/QUAIU#1	E 11	Durgapura	26 °51'N	75 ° 47'E	390
WH1283	G 12	P13352/PBW343//WH711/3/PBW550	E 12	Sriganganagar	29 ° 66'N	75 ° 53'E	175.6
HD3086	G 13	DBW14/HD2733//HUW468	E 13	Tabiji	26 ° 35'N	74 ° 61'E	508
			E 14	Nagina	29 ° 28' N	78 ° 32'E	245
			E 15	Bulandshahr	28 ° 40'N	77 ° 84'E	195
			E 16	Modipuram	29 °05' N	77 °70'E	226
			E 17	Ujhani	28 ° 06' N	79 ° 34' E	
			E 18	Pantnagar	29 ° 02'N	79 ° 48'E	243.8
			E 19	Kashipur	29 ° 21'N	78 ° 96'E	218

Table 2: AMMI analysis of wheat genotypes evaluated in nineteen environments

Source	Degree of freedom	Mean Sum of Squares	Significance level	Proportional contribution of factors	GxE interaction Sum of Squares (%)	Cumulative Sum of Squares (%) by IPCA's
Treatments	246	219.76	***	81.4559		
Genotype (G)	12	607.63	***	10.9864		
Environment (E)	18	1414.01	***	38.3499		
GxE interaction	216	98.69	***	32.1196		
IPC1	29	292.38	***		39.78	39.78
IPC2	27	111.10	***		14.07	53.85
IPC3	25	106.91	***		12.54	66.38
IPC4	23	82.05	***		8.85	75.24
IPC5	21	78.58	***		7.74	82.98
IPC6	19	70.69	***		6.30	89.28
IPC7	17	44.77	***		3.57	92.85
Residual	55	27.72	***			
Error	741	16.61				
Total	987	67.24				

doi: 10.5958/2582-2683.2023.00013.8

pointed towards, G9, G6, G7 as high yielders. Consistent yield of G11, G4, G13 as per least values of standard deviation more over the values of CV identified G11, G4, G13, genotypes for the consistent yield performance for NEPW zone of the country. More over the values of BLGM favored G9, G6, G7. The BLUP-based simultaneous selections, such as HMGV identified G9, G6, G13, while values of RPGV favored G9, G6, G13 and HMRPGV settled for G9, G6, G13 genotypes. The evaluation of adaptability and stability of wheat genotypes through these BLUP-based indices was reported by Pour-Aboughadareh *et al.,* 2019. The estimates of HMGV, RPGV, and HMRPGV had the same genotype ranking that was reported Anuradha *et al.,* 2022.

Non parametric measures

These measures consider the ranks of genotypes as per their corrected yield across environments. S_i^1 values pointed for G3, G13, G12 while S_i^2 selected G3, G13, G12 and values of S_i^3 favoured G3, G13, G4 as desirable genotypes (Table 4). G3, G13, G12 selected by values of S_i^4 & measure S_i^5 pointed towards G3, G13, G2 while S_i^6 observed suitability of G3, G13, G12 and lastly S_i^7 values identified G3, G13, G4 genotypes (Table 4). The mentioned strategy determines the stability of genotype over environment if its rank is similar over other environments (biological concept). Nonparametric measures of phenotypic stability were associated with the biological concept of stability (Vaezi *et al.,* 2018). Non parametric composite measures $NP_i^{(1)}$ to $NP_i^{(4)}$, consider the ranks of genotypes as per their yield and corrected yield across environments simultaneously. $NP_i^{(1)}$ measure observed suitability of G3, G13, G12 whereas as per $NP_i^{(2)}$, genotypes G12, G3, G10 would be of choice while $NP_i^{(3)}$ identified G3, G10, G12. Last composite measure $NP_i^{(4)}$ found G3, G12, G10 as genotypes of choice for this zone.

Biplot analysis

The first two significant PC's has explained about 65.4 per cent of the total variation in the AMMI, BLUP and non parametric measures considered for this study (Table 5) with

Table 3: AMMI along with BLUP based measures of yield for wheat genotypes

Code	Mean	IPC1	IPC2	IPC3	IPC4	IPC5	IPC6	IPC7	MASV1	MASV	ASV1	ASV	BLAvg	BLStdev	BLCV	BLGM	BLHM
G 1	58.71	1.582	-0.097	0.714	-0.041	1.249	-0.665	0.376	5.12	3.55	4.47	2.66	58.58	6.03	10.29	58.27	57.96
G 2	58.41	0.462	1.277	-2.067	-1.740	0.992	2.007	-0.206	5.72	5.40	1.83	1.49	58.34	5.93	10.16	58.05	57.75
G 3	57.24	-0.914	0.139	-0.033	1.750	1.341	0.975	-0.323	4.43	3.81	2.59	1.54	57.36	6.79	11.84	56.96	56.56
G 4	58.74	1.371	-0.458	-1.253	-0.769	0.325	-2.138	-1.892	5.58	4.64	3.90	2.35	58.59	4.90	8.37	58.39	58.19
G 5	55.67	-2.593	-1.522	-0.349	0.845	-0.806	-0.674	-1.105	8.03	5.39	7.49	4.62	55.87	7.50	13.43	55.41	54.96
G 6	61.82	0.880	2.487	0.899	1.375	-2.831	0.940	-0.934	7.00	6.43	3.52	2.89	61.55	7.69	12.49	61.08	60.60
G 7	58.76	1.304	-0.352	0.718	-0.423	-0.866	-0.331	2.274	4.80	3.70	3.70	2.22	58.87	5.22	8.86	58.65	58.42
G 8	53.61	-4.838	0.262	0.817	-2.099	-0.671	-0.347	0.493	14.17	8.88	13.68	8.14	53.98	10.18	18.85	52.99	51.90
G 9	64.92	1.615	2.126	1.219	-1.296	0.404	-1.280	-0.047	6.47	5.19	5.04	3.45	64.12	6.87	10.71	63.75	63.35
G 10	55.95	1.614	-3.019	2.534	-0.248	-0.073	1.004	-0.482	7.90	6.64	5.47	4.06	56.43	7.71	13.67	55.89	55.30
G 11	57.86	0.711	-0.999	-2.862	1.071	-1.261	-0.844	1.345	6.34	5.68	2.24	1.56	57.84	4.29	7.42	57.69	57.53
G 12	56.62	-1.484	1.120	0.617	2.152	2.053	-0.440	0.606	6.59	5.46	4.34	2.74	56.79	7.83	13.79	56.28	55.76
G 13	59.04	0.290	-0.965	-0.953	-0.576	0.143	1.793	-0.103	3.22	3.16	1.27	1.08	59.00	5.06	8.58	58.80	58.59

Table 4: Non parametric measures of yield for wheat genotypes

Code	S_i^1	S_i^2	S_i^3	S_i^4	S_i^5	S_i^6	S_i^7	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$	PRVG	MHPRVG
G 1	4.503	14.813	0.233	3.849	3.346	9.664	4.194	3.316	0.553	0.571	0.668	1.005	1.003
G 2	4.140	12.673	0.228	3.560	2.931	8.331	4.096	2.895	0.482	0.537	0.624	1.003	0.998
G 3	3.462	8.953	0.201	2.992	2.349	6.190	3.611	2.316	0.289	0.389	0.451	0.983	0.980
G 4	4.444	14.444	0.226	3.801	3.368	9.143	4.063	3.316	0.553	0.543	0.635	1.008	1.004
G 5	5.076	19.497	0.260	4.416	3.945	10.627	4.683	3.842	0.384	0.518	0.595	0.958	0.952
G 6	4.690	16.175	0.260	4.022	3.274	8.628	4.680	3.263	1.088	0.859	1.001	1.055	1.050
G 7	4.526	14.982	0.230	3.871	3.435	8.986	4.132	3.421	0.489	0.561	0.656	1.012	1.009
G 8	5.427	22.544	0.283	4.748	4.194	13.518	5.092	4.053	0.450	0.547	0.625	0.924	0.901
G 9	4.304	13.579	0.235	3.685	3.047	7.857	4.222	2.947	1.474	1.373	1.603	1.101	1.096
	4.257	13.099	0.233	3.619	2.953	8.137	4.203	2.947	0.327	0.414	0.487	0.968	0.958
G 11	4.842	17.211	0.245	4.149	3.690	10.168	4.419	3.632	0.404	0.559	0.652	0.997	0.992
G 12	4.117	12.450	0.229	3.528	2.859	7.068	4.126	2.842	0.284	0.409	0.477	0.972	0.967
G 13	3.509	9.205	0.201	3.034	2.410	6.304	3.618	2.368	0.395	0.461	0.533	1.014	1.012

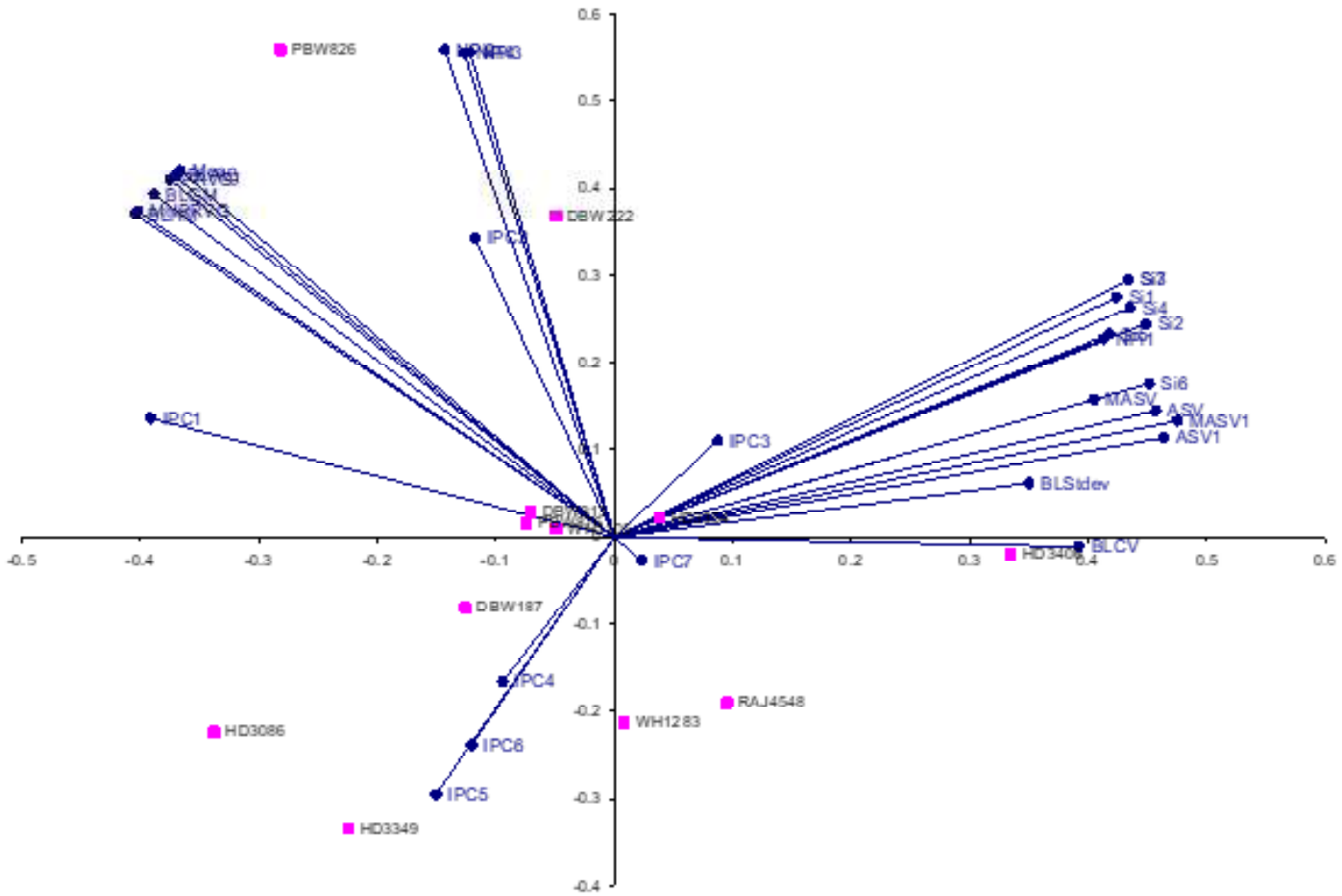

Figure 1: Biplot analysis of AMMI, BLUP and non parametric measures

Table 5: Loadings of AMMI, BLUP and non parametric measures

Measure	PC1	PC2	Measure	PC1	PC2
Mean	-0.190	0.251	BLGM	-0.201	0.235
IPC1	-0.202	0.081	BLHM	-0.209	0.222
IPC2	-0.061	0.205	PRVG	-0.194	0.246
IPC3	0.045	0.066	MHPRVG	-0.208	0.224
IPC4	-0.049	-0.099	S_i^1	0.219	0.165
IPC5	-0.078	-0.176	$S_i^{2,}$	0.232	0.146
IPC6	-0.062	-0.143	S_i^3	0.224	0.176
IPC7	0.012	-0.016	S_i^4	0.225	0.158
MASV1	0.245	0.080	S_i^5	0.216	0.139
MASV	0.209	0.094	S_i^6	0.233	0.105
ASV1	0.239	0.068	S_i^7	0.224	0.176
ASV	0.236	0.087	$NP_i^{(1)}$	0.213	0.136
BLAvg	-0.192	0.248	$NP_i^{(2)}$	-0.074	0.334
BLStdev	0.181	0.037	$NP_i^{(3)}$	-0.063	0.333
BLCV	0.202	-0.006	$NP_i^{(4)}$	-0.065	0.332
				72.73	46.75	25.97

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00013.8

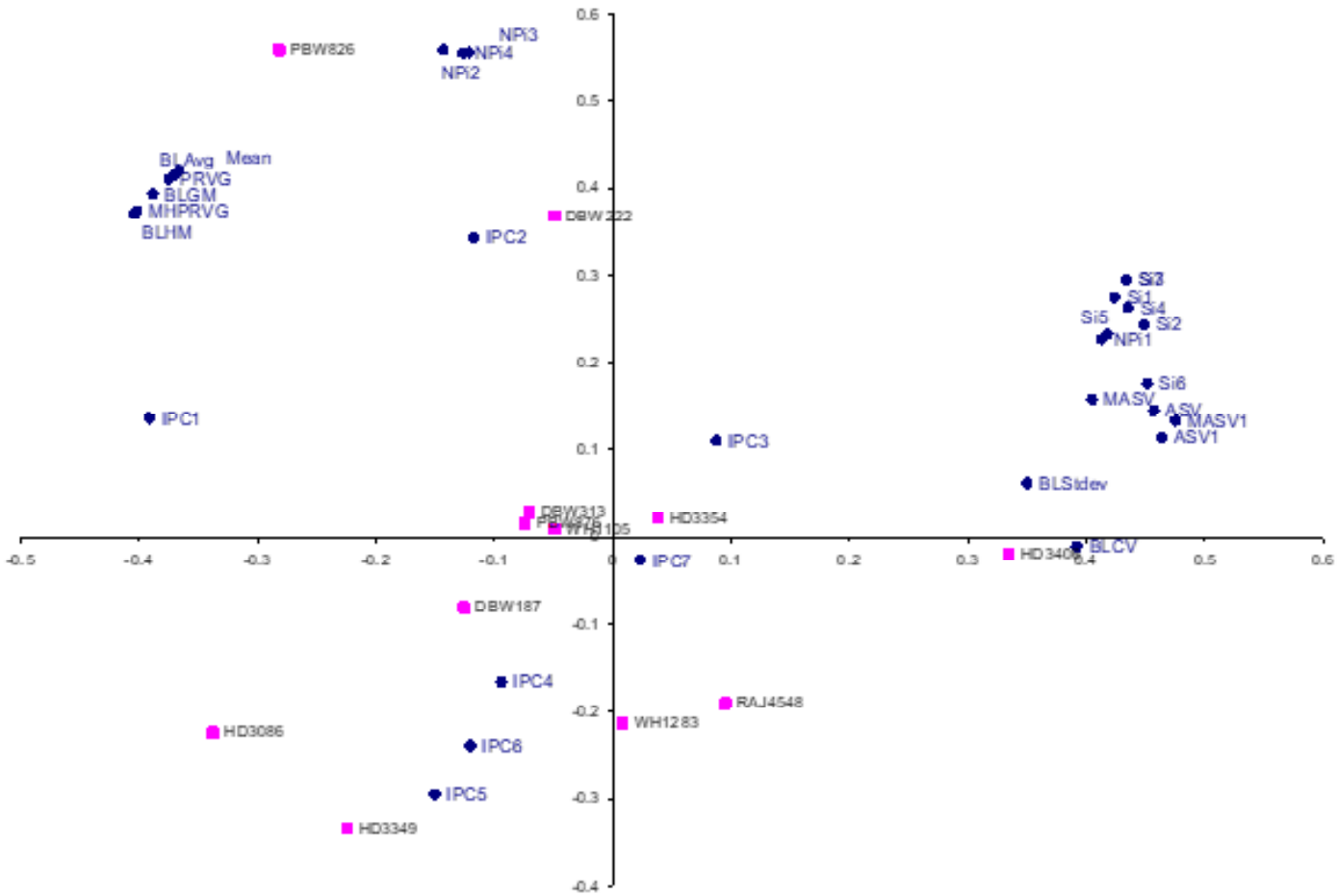

Figure 2: Clustering pattern of AMMI, BLUP and non parametric measures

respective contributions of 35.4 per cent & 29.9 per cent by first and second principal components respectively (Ahakpaz *et al.*, 2021). Measures S_i^1, S_i^2, S_i^4, S_i^3, $NP_i^{(1)}$, S_i^6, ASV, ASV1, IPC7 accounted more of share in PC1 whereas RPGV HMRPGV, HMGV, BLAvg, BLGM, $NP_i^{(4)}$ contributed more in PC2. The association analysis among measures had been explored with the biplot analysis. In the biplot vectors of measures expressed acute angles would be positively correlated whereas those achieved obtuse or straight line angles would be negatively correlated. Independent type of relationships had expressed by right angles between vectors. Very tight positive relationships observed for IPC4, IPC6, IPC5 in one quadrants. IPC7 showed affinity with BLCV measure. BLUP based measures expressed very tight relationships among themselves as well with IPC1. Large group ASV, ASV1, MASV, MASV1, $NP_i^{(1)}$, S_i^1 to S_i^7, BLStd observed in separate quadrant. Measure $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$ expressed tight bondage with moderate degree with IPC2. This group expressed right angles with group of BLUP based measures. Similarly group of IPC4, IPC6, IPC5 maintained right angle with $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$ (Fig. 1). Measures IPC2, $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$ formed a cluster adjacent to another cluster of BLUP based measures in same quadrant. Measures ASV, ASV1, MASV, MASV1, clustered with S_i^1, S_i^2, S_i^3, S_i^4, S_i^5, S_i^6, S_i^7 BLStd and $NP_i^{(1)}$, BLCV observed with IPC7 in different quadrant of biplot analysis. Small cluster of IPC4, IPC5, IPC63 placed in a cluster (Fig. 2).

Association analysis

Average yield had expressed direct and indirect relationships with measures (Table 6). Notably positive values for ASV, ASV1, MASV, MASV1, perfect positive correlation with BLAvg, BLGM, HMGV, RPGV, HMRPGV and negative values for with $NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$. AMMI based measures ASV & ASV1 showed moderate to strong positive correlations with measures while weak negative with ($NP_i^{(2)}$,

Table 6: Spearman rank Correlation analysis among measures of wheat genotypes

	IPC1	IPC2	IPC3	IPC4	IPC5	IPC6	IPC7	MASV1	MASV	ASV1	ASV	BLAvg	BLStdev	BLCV	BLGM	BLHM	PRVG	MHPRVG	S_i^1	$S_i^{2,}$	S_i^3	S_i^4	S_i^5	S_i^6	S_i^7	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	$NP_i^{(4)}$
Mean	-0.593	-0.390	-0.071	0.137	0.088	0.060	0.000	0.522	0.495	0.478	0.401	1.000	0.473	0.571	1.000	1.000	1.000	1.000	0.203	0.203	0.269	0.203	0.247	0.335	0.269	0.264	-0.739	-0.588	-0.632
IPC1		-0.011	0.363	-0.170	-0.016	-0.209	-0.071	-0.209	-0.192	0.016	-0.016	-0.593	-0.286	-0.368	-0.593	-0.593	-0.593	-0.593	-0.104	-0.104	-0.082	-0.104	-0.077	-0.132	-0.082	-0.027	0.585	0.505	0.555
IPC2			0.258	-0.077	0.264	0.077	0.121	0.049	0.121	-0.104	0.016	-0.390	0.313	0.231	-0.390	-0.390	-0.390	-0.390	-0.115	-0.115	0.016	-0.115	-0.242	-0.242	0.016	-0.275	0.420	0.379	0.357
IPC3				-0.038	-0.099	0.071	0.027	0.407	0.286	0.599	0.659	-0.071	0.692	0.626	-0.071	-0.071	-0.071	-0.071	0.110	0.110	0.396	0.110	0.011	-0.110	0.396	0.049	0.179	0.280	0.242
IPC4					0.055	-0.033	0.000	-0.038	0.011	-0.148	-0.060	0.137	0.126	0.165	0.137	0.137	0.137	0.137	-0.154	-0.154	-0.071	-0.154	-0.258	-0.247	-0.071	-0.253	-0.486	-0.308	-0.291
IPC5						0.066	0.011	-0.335	-0.352	-0.027	-0.242	0.088	0.104	0.137	0.088	0.088	0.088	0.088	-0.725	-0.725	-0.626	-0.725	-0.637	-0.538	-0.626	-0.621	-0.272	-0.434	-0.418
IPC6							-0.044	-0.203	0.022	-0.423	-0.368	0.060	0.148	0.165	0.060	0.060	0.060	0.060	-0.467	-0.467	-0.335	-0.467	-0.560	-0.473	-0.335	-0.549	-0.382	-0.423	-0.456
IPC7								-0.159	-0.044	-0.082	-0.154	0.000	-0.071	-0.093	0.000	0.000	0.000	0.000	0.104	0.104	0.088	0.104	0.137	0.071	0.088	0.132	-0.080	0.220	0.170
MASV1									0.857	0.714	0.874	0.522	0.731	0.709	0.522	0.522	0.522	0.522	0.577	0.577	0.824	0.577	0.484	0.473	0.824	0.489	-0.096	0.088	0.044
MASV										0.379	0.588	0.495	0.593	0.560	0.495	0.495	0.495	0.495	0.412	0.412	0.643	0.412	0.302	0.313	0.643	0.302	-0.179	-0.005	-0.049
ASV1											0.934	0.478	0.676	0.681	0.478	0.478	0.478	0.478	0.478	0.478	0.621	0.478	0.505	0.451	0.621	0.538	-0.003	0.115	0.082
ASV												0.401	0.764	0.742	0.401	0.401	0.401	0.401	0.577	0.577	0.786	0.577	0.522	0.456	0.786	0.544	0.052	0.209	0.170
BLAvg													0.473	0.571	1.000	1.000	1.000	1.000	0.203	0.203	0.269	0.203	0.247	0.335	0.269	0.264	-0.739	-0.588	-0.632
BLStdev														0.989	0.473	0.473	0.473	0.473	0.115	0.115	0.467	0.115	-0.005	-0.016	0.467	0.011	-0.288	-0.159	-0.220
BLCV															0.571	0.571	0.571	0.571	0.099	0.099	0.429	0.099	-0.005	-0.005	0.429	0.011	-0.393	-0.264	-0.324
BLGM																1.000	1.000	1.000	0.203	0.203	0.269	0.203	0.247	0.335	0.269	0.264	-0.739	-0.588	-0.632
BLHM																	1.000	1.000	0.203	0.203	0.269	0.203	0.247	0.335	0.269	0.264	-0.739	-0.588	-0.632
PRVG																		1.000	0.203	0.203	0.269	0.203	0.247	0.335	0.269	0.264	-0.739	-0.588	-0.632
MHPRVG																			0.203	0.203	0.269	0.203	0.247	0.335	0.269	0.264	-0.739	-0.588	-0.632
S_i^1																				1.000	0.863	1.000	0.962	0.929	0.863	0.956	0.349	0.577	0.544
$S_i^{2,}$																					0.863	1.000	0.962	0.929	0.863	0.956	0.349	0.577	0.544
S_i^3																						0.863	0.747	0.703	1.000	0.753	0.206	0.500	0.445
S_i^4																							0.962	0.929	0.863	0.956	0.349	0.577	0.544
S_i^5																								0.956	0.747	0.995	0.321	0.511	0.489
S_i^6																									0.703	0.962	0.288	0.445	0.423
S_i^7																										0.753	0.206	0.500	0.445
$NP_i^{(1)}$																											0.321	0.511	0.489
$NP_i^{(2)}$																												0.904	0.926
$NP_i^{(3)}$																													0.995

$NP_i^{(3)}$, $NP_i^{(4)}$). (Anuradha *et al.*, 2022). Measures considered all significant IPC's showed moderate to strong positive correlation values (S_i^1, S_i^2, S_i^3, S_i^4, S_i^5, S_i^6, S_i^7 $NP_i^{(1)}$ $NP_i^{(2)}$ $NP_i^{(3)}$, $NP_i^{(4)}$) along with a few very weak negative values also. BLUP based measures expressed strong to moderate negative correlations with non parametric measures ($NP_i^{(2)}$, $NP_i^{(3)}$, $NP_i^{(4)}$). S_i^s exhibited only moderate to strong positive values with other measures with only weak negative values with IPCs (Pour Aboughadareh *et al.*, 2022). Lastly composite non parametric measures expressed both type of relationships with other measures. Mostly positive values expressed by $NP_i^{(1)}$ contrasting to few of negative values by $NP_i^{(2)}$ $NP_i^{(3)}$, $NP_i^{(4)}$.

CONCLUSION

The study concludes that the AMM1 analysis showed existence of significant variations due to environment, G×E interaction and the genotypes. Based on MASV1 values, the wheat genotypes G13, G3 and G7 were rated as stable yielders.

ACKNOWLEDGEMENTS

Authors sincerely acknowledge the training by Dr J Crossa and financial support by Dr. A.K Joshi & Dr RP Singh CIMMYT, Mexico along with hard work of the staff at coordinating centers of AICW&BIP project to carry out the field evaluation of genotypes and data recording.

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Online Manuscript No. 97394 recived on 30.3.2022
Manuscript accepted for publication on 5.11.2022

Journal of Eco-friendly Agriculture **18**(1): 80-85. 2023
doi: 10.5958/2582-2683.2023.00014.X

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Evaluation of variability, heritability and genetic advance for yield and yield components in linseed (*Linum usitatissimum* L.) genotypes

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ABSTRACT

Eighteen genotypes of linseed (*Linum usitatissimum* L.) were evaluated for genetic variability, heritability and genetic advance for yield and yield components. Its yield can be increased with the knowledge of the relationship among yield components is essential for the formulation of breeding programmes. Both phenotypic and genotypic coefficients of variation (PCV and GCV) were highest for seed yield per acre followed by the number of capsules per plant (41.57 and 40.28, respectively). On the other hand, parameters such as plant stand per plot and oil content showed a minimal phenotypic and genotypic coefficient of variation. Estimates of heritability were highest for days to maturity (98.54%), days to 50% flowering (97.44%) and yield per acre (93.86%). It was the minimum for plant stand per acre and oil content. Similarly, the genetic advance was highest for yield per acre and lowest for oil content percentage. The highest heritability for the traits like days to maturity, days to 50% flowering and yield per acre give a positive response to the selection. Traits having high heritability and high genetic advance are supposed to be under the control of additive genes; hence, these can be improved by selection based on phenotypic performance. Invariably higher PCV as compared to GCV indicated the role of environment in the expression of the traits.

Key words: Variability, phenotypic coefficient variation, genotypic coefficient of variation, heritability, genetic advance

Linseed (*Linum usitatissimum* L. 2n=30) is an important winter (*rabi*) oilseed crop grown after rapeseed and gobhi sarson. It is one of the oldest crops under cultivation as an annual, self-pollinating plant species belonging to the family Linaceae genus *Linum* commonly known as "Alsi" and is assumed to be originated in southwest Asia, particularly in India (Vavilov, 1935 and Richharia, 1962). Linseed crop is grown for seed oil, stem fibre and to a lesser extent, flour. Its seeds are used in the treatment of some challenging human and animal diseases. The crop is mainly cultivated in the states of Rajasthan, West Bengal, Karnataka, Orissa, Bihar, Chhattisgarh Madhya Pradesh, Uttar Pradesh Maharashtra and Punjab. Its oil is used for paints, inks, varnish and other wood treatments, soap, linoleum, putty and pharmaceuticals.

The fibre from flax/linseed is used as valuable raw material for textiles, thread/rope and packaging materials. The straw and short fibre are used for producing pulp to make special papers: for cigarettes, currency notes and artwork; and the wooden part serves as biomass energy or litter in cattle farming (Rowland, 1998). Knowledge of the relationship among yield components is essential for the formulation of breeding programmes.

Genetic variability and heritability are very much important and useful ways for selection. Genetic variability tells us about a great deal of variation prevails in our existing germplasm and heritability provides us with how many traits are transmitted from parents to offspring. These parameters help us to evaluate the genotypic and environmental effects to achieve better selection. Through estimates of heritability, we are able to calculate genetic advance, because without genetic advance heritability is not much effective approach to selection (Rajaravindran *et al*., 2000; Kadir *et al*., 1996). The present study was conducted to evaluate linseed genotypes for their yield and yield-contributing parameters with the objective to accumulate knowledge of this crop and generating selection strategies to enhance yield.

MATERIALS AND METHODS

The experiment comprising eighteen linseed genotypes was laid out in a Randomized Block Design with three replications at Punjab Agricultural University, Regional Research Station, Gurdaspur, India. The plot size for each entry was 5 x 3 m^2 following 25 cm spacing. Heritability in broad sense was estimated as suggested by Burton and

Devane (1952). The expected genetic advance at 5% selection intensity was calculated by the formula used by Johnson *et al.* (1955). The software was used for the analysis of heritability, genetic variability and genetic advance parameters.

RESULTS AND DISCUSSION

The plant breeding programme's primary objective is to develop high-yielding superior genotypes/lines superior to existing ones through genetic manipulation. Linseed is an important *rabi* oilseed crop grown in India and other developing countries of the world.

The present study was carried out with subject to different genetical studies *viz*., genetic variability, heritability and genetic advance laid the foundation for studies, assessment and ranking of superior genotypes are identified and tested in multilocation trials to be used in further breeding programmes. The genetic estimates of variability such as range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense (H), Genetic advance (GA) and genetic advance as percentage of mean (GA % M) are presented in Table 1 which were computed to have a better understanding of the nature of the gene action for various quantitative characters for an effective breeding programme.

Range

The range indicated a broad base of variation among the 16 genotypes for all the characters except for the number of seeds per capsule (4.66- 6.73) and 1000 seed weight (6.10-10.6). A wide range of variation was also found for all or almost all these characters by Satapathi *et al.* (1987) and Rao (2007). However, broad range was observed for days to 50% flowering, days to maturity, the number of capsules per plant and yield per acre. Similarly, Gupta *et al.* (1999) reported broad range for the number of primary branches, capsules and seed yield only.

Phenotypic and genotypic coefficients of variation (PCV and GCV)

The phenotypic coefficient of variation (PCV) ranged from 3.25 per cent for oil content to 41.57 per cent for yield per acre (Table 1 & Fig. 1). The PCV estimates showed that the phenotypic variability was low (below 10%) for oil content, days to maturity, plant stand per plot and days to 50 per cent flowering. Moderate PCV (10-20%) for the number of seeds per capsule (17.30) was recorded. High PCV (above 20%) for seed weight, the number of seeds per capsule and yield per acre was recorded. The values of the genotypic coefficient of variation (GCV) demonstrated a similar pattern of PCV for all eight characters and ranged from 1.07 to 40.28. Bibi *et al.* (2013) in linseed showed high potential for effective

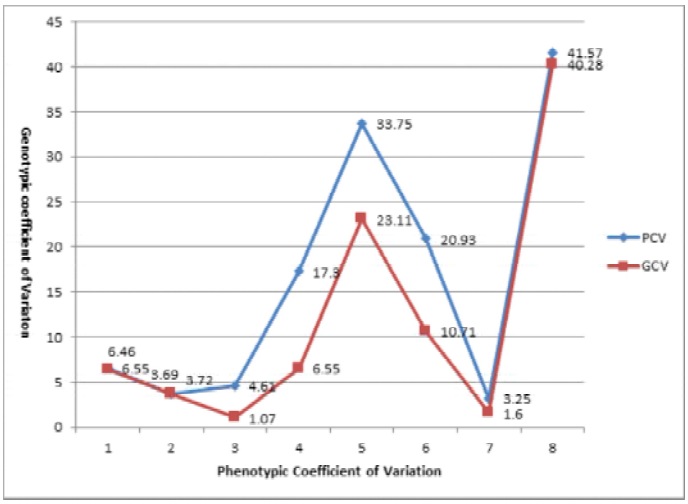

Fig. 1: Phenotypic and genotypic coefficients of Linseed
[1. Days to flowering, 2. Days to maturity, 3. Plant stand per plot, 4. Number of seeds per capsule, 5. Number of capsules per plant, 6. Seed weight of 1000 seeds, 7. Oil content in percentage, 8. Seed yield per acre]

Table 1. General Mean, Range, PCV (%), GCV (%), Heritability h^2 % (BS), Genetic Advance GA %

Character	Range	General Mean	PCV (%)	GCV (%)	h^2 (%)	GA	GAM	CD (5%)	CV (%)
DTF	100.33-115.66	107.33	6.55	6.46	97.44	14.12	13.15	1.87	1.04
DTM	149.64-164.33	156.66	3.72	3.69	98.54	11.83	7.55	1.17	0.44
PSP	700.80-781.66	735.55	4.61	1.07	5.41	3.78	0.51	55.02	4.49
NOSC	4.66- 6.73	5.75	17.30	6.55	14.35	0.29	5.11	1.53	16.01
NOCP	16.46- 57.13	32.15	33.75	23.11	46.87	10.47	32.59	13.17	24.60
SW	6.10- 10.6	8.04	20.93	10.71	26.16	0.90	11.28	2.41	17.99
OC	34.99- 38.12	36.42	3.25	1.60	24.18	0.59	1.62	1.71	2.83
YA	191.11- 678.89	452.08	41.57	40.28	93.86	363.47	80.39	77.54	10.29

DTF- Days to flowering, DTM- Days to maturity, PSP- Plant stand per plot, NOSC- Number of seeds per capsule, NOCP- Number of capsules per plant, SW- Seed weight of 1000 seeds, OC- Oil content in percentage, YA- Seed yield per acre.

selection of seed yield per acre. Similar to these findings Paul *et al.* (2017) also observed that the PCV values were greater than the GCV values for all the traits studied.

The present study showed that the PCV estimates were higher than the GCV estimates because the former includes the variation due to environment as well as variation due to interactions. Hence, the selection for these characters sometimes may be misleading. These environmental factors could be due to the heterogeneity in soil fertility status and other unpredictable factors [Reddy *et al.* (2012) in Okra, Khan *et al.* (2007) in Sesame and Manggoel *et al.* (2012) in Cowpea]. A similar pattern of PCV and GCV was reported by several workers for all or most of these characters (Satapathi *et al*., 1987; Rao, 2007; Basavaraj *et al.,* 2011 and Asgarinia *et al*., 2014) for high PCV and GCV for the number of capsules per plant, the number of seeds per capsule and yield per plant. Chauhan *et al.* (2012) reported similar characters of high PCV and GCV. The difference among the GCV and PCV values for different characters pointed towards the effect of the environment in expressing the variation in characters. If the difference is the least it means the environment is much affecting the variable performance of the characters, but if the difference is more, means there is much influence of environment in the expression of the characters. The success of improvement in any character is based on phenotypic selection, which depends upon the correspondence between phenotype and genotype. Hence, the selection intensity in a population relies upon the amount of heritable variation present in the population.

Heritability

Heritability is the main parameter for selecting genotypes that permits greater effectiveness of selection by separating the environmental impact from the total variability. The heritability is classified as low (below 30%), medium (30-60%) and high (above 60%). The plant breeders can select genotypes from a diverse genetic population through estimates of heritability. Therefore, high heritability helps in the effective selection of a particular character. In the present study, the heritability in broad sense (h^2) ranges from 5.41 per cent for plant stand per plot to 98.54 per cent days to maturity (Table 1 and Fig. 2).

Minimum heritability estimates (5.41) were for plant stand followed by the number of seeds per capsule (14.35%), tagged on by 1000 seed weight (26.16%) and oil content (24.18%) which means that the effect of environmental conditions is more on plant stand as compared to other traits. The traits with low heritability possession are difficult to improve through phenotypic selection due to the masking influence of the environment on the genotypic effect.

A moderate estimate of heritability (30-60%) was found in the number of capsules per plant (46.87%). Khan and Gupta (1995) and Tadesse *et al.* (2010) though accounted for a moderate estimate of heritability for plant height trait. Even Thakur *et al.* (2020) reported moderate heritability for characters *viz*., days to 50% flowering (51.77%), seeds per capsule (49.80%) and harvest index (37.51%). They also affirmed that the phenotypic variability in these characters had a greater share of environmental factors. Further, Tefra and Gumru (2020) also showed moderate heritability for plant height (36.23%), primary branch (40.00%) per plant and secondary plant per plant (30.12%). Also, similar findings were reported by Kumar *et al.* (2016) as heritability is a good catalogue for the transmission of characters from parents to their progeny.

High estimates of heritability were for days to maturity (98.54%), days to 50 per cent flowering (97.44%) and seed yield per plot (93.86%). The character which exhibited high heritability indicates the presence of additive gene action and such characters could be fixed by resorting to selection. Therefore, high heritability helps in the effective selection of a particular character. Terfa and Gumru (2020) reported the highest broad sense heritability value for the harvest index (77.78%) followed by yield per hectare (73.21%). The result for seed yield per plant disagreed with the report of Upadhyay *et al.* (2019). Kumar *et al.* (2016) reported similar results as heritability is a good index of transmission of characters from parents to their progeny. High estimates of heritability similarly were reported by earlier workers (Gupta and Godawat, 1981; Satapathi *et al.,* 1987; Rao, 2007; Pali and Meheta, 2013) for seed weight. Also, these results are in accordance with the findings of Chauhan *et al.* (2012) who observed high heritability along with high genetic advance for plant height (cm), seeds per capsule, capsules per plant, number of primary branches per plant and number of secondary branches per plant. Tewari *et al.* (2012) showed high heritability with high genetic advance for capsules per plant and seed yield per plant.

Genetic advance

The effective and efficient selection progress based on genetic advance is a useful indicator for the selection of the base population. In the present study genetic advance as a percent mean was categorized as low (0-10%), moderate (10-20%) and high (e"20%) as given by Johnson *et al.* (1955) and Falconer and Mackay (1996). In the present study, the GA % M ranged from 0.51% for plant stand per plot to 80.39% yield per plot.

The low genetic advance was recorded for plant stand per plot (0.51), followed by oil content (1.62), in perusal with

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00014.X

the number of seeds per capsule (5.11) and days to maturity (7.55). Dhirhi and Mehta (2019) reported characters such as days to 50 per cent flowering, days to maturity, and 1000 seed weight (g) were for low genetic advance mean as percentage. Similarly, the minimum value of genetic advance in percent of the mean has been noted for the number of seeds per capsule (0.29%) as reported by Kasana *et al.* (2018). Likewise, Muduli and Patnaik (1993) and Naik and Satapathy (2002) observed low genetic advance for seed weight, plant height and days to 50% flowering.

The moderate genetic advance percent was accounted for 1000 seed weight (11.28%) and days to 50 per cent flowering (13.15%). The moderate genetic advance was stated for the number of primary branches per plant (36.02%), the number of secondary branches per plant (28.61), plant height (cm) (21.06%) total number of branches per plant (16.93%) by (Dhirhi and Mehta 2019). Yadav and Singh (2016) reported high heritability in broad sense for all the characters except days to maturity which showed moderate heritability.

Highest genetic advance percentage mean for yield per plot was 80.39 per cent. Similarly, the number of capsules per plant recorded the highest genetic advance as a percent of mean (66.59) (Dhirhi and Mehta 2019). Kasana *et al.* (2018) observed the maximum value of genetic advance in percent of mean has been observed for the number of capsules per plant (41.75%) but contradicts other observations of maximum genetic advance for plant height (15.68%), days to flowering (13.58%), days to maturity (7.27%), size of corolla (4.45%), oil content (3.68%), seed yield per plant (1.72%), 1000-seed weight (0.54%), capsule size (0.43%) and the number of primary branches per plant (0.40%). Also, Yadav and Singh (2016) recorded high genetic advance for the number of capsules per plant and other characters showed moderate to low genetic advance.

The characters showed high genetic advance indicating that the character is governed by additive genes and selection will help to improve such traits. The traits having moderate to low genetic advance indicate that the character is governed by non-additive genes. Similar results were also found by Dash *et al.* (2016). Similarly, the high genetic advance was accounted for by Vijayakumar and Rao (1975) and Gupta and Godawat (1981) for branches per plant, capsules per plant, yield per plant and seed weight. Also, high genetic advance for the number of primary branches per plant as observed by Naik and Satapathy (2002).

Heritability and Genetic advance

Low heritability along with low genetic advance is manifested for plant stand per plot (5.41 and 0.51%, respectively) (Table 1). Similarly, low heritability with low genetic advance was found for the number of seeds per capsule as observed Pali and Meheta (2013).

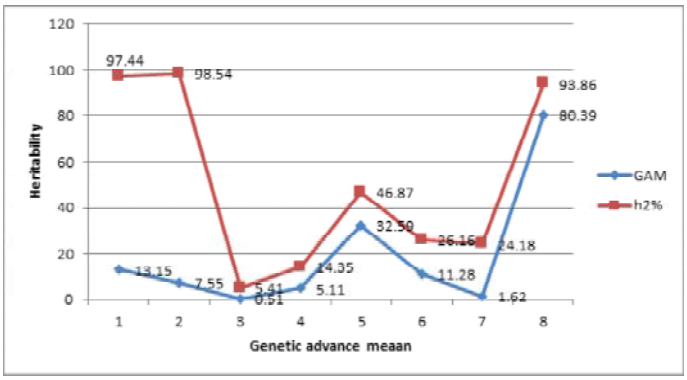

Fig. 2: Genetic advance mean and Heritability of Linseed
[1. Days to flowering, 2. Days to maturity, 3. Plant stand per plot, 4. Number of seeds per capsule, 5. Number of capsules per plant, 6. Seed weight of 1000 seeds, 7. Oil content in percentage, 8. Seed yield per acre]

Moderate heritability (46.87) coupled with moderate GA % M (32.59) was observed for the number of capsules per plant indicating that this character seems to be heritable and can be improved by selection (Table 1, Fig. 2). Other than this moderate heritability (24.18) with low GA % M (1.62) was observed for oil content, but low genetic advance for plant height. Pali and Mehata (2013) reported high heritability coupled with moderate genetic advance for days to flower, days to maturity and 1000-seed weight and plant height. Similarly, Vardhan and Rao (2012) accounted for high heritability with high genetic advance for 1000-seed weight. Likewise, Kanwar *et al.* (2014) observed high heritability coupled with high genetic advance for the number of capsules per plant, seed yield per plant, biological yield, plant height and the number of primary branches per plant. Thus, characters with higher heritability and genetic advance can be improved by adopting a selection scheme capable of exploiting both additives as well as non-additive genetic components. Therefore, the selection of traits contributing to yield is an important aspect.

Also, Chauhan *et al.* (2012) observed high heritability along with high genetic advance for plant height (cm), seeds per capsule, capsules per plant, number of primary branches per plant and number of secondary branches per plant.

CONCLUSION

- In conclusion, it is clear that the significant genetic variability in any breeding material is a prerequisite as it not only provides a basis for selection but also provides some valuable information regarding the

selection of diverse parents for use in the hybridization programme.

- The highest genetic advance and heritability for the traits like days to 50 per cent flowering and yield per acre give a positive response to the selection.
- Traits having high h^2 and high GA are supposed to be under the control of additive genes; hence, these can be improved by selection based on phenotypic performance.
- Invariably higher PCV as compared to GCV indicated the role of the environment in the expression of the traits.

ACKNOWLEDGEMENT

We are thankful to All India Coordinated Linseed Research Project, Kanpur for providing all necessary facilities and support during the course of the investigation.

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Online Manuscript No. 97339 recived on 24.3.2022
Manuscript accepted for publication on 18.11.2022

Journal of Eco-friendly Agriculture **18**(1): 86-92. 2023
doi: 10.5958/2582-2683.2023.00015.1

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Effect of edible coating on shelf life and market acceptability of Guava (*Psidium guajava* L.*) cv. Lucknow-49*

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ABSTRACT

The studies conducted with a view to obviating the postharvest losses in guava by extending its shelf life through beeswax, edible oil and cornstarch coatings revealed minimum physiological loss in weight and decaying per cent (44.44), maximum flesh firmness (4.20 Kg/cm²) and maximum economical return (₹ 45 kg^{-1}) of fruit coated with cornstarch (2%) + sunflower oil (1%) + beeswax (1%) upto 12th day of storage.

Keywords: Guava, cornstarch, edible coating, oil, shelf life, bee wax

Guava (*Psidium guajava* L.) known as the "apple of tropics" belongs to the family Myrtaceae. The total area, production and productivity of guava in India is about 2.60 million hectare with 3826 metric tonnes production and 15.93 metric tonnes ha^{-1}, respectively. In Madhya Pradesh, total area, production and productivity of guava is 28.44 thousand hectare, 990 thousand million tonnes and 34.81 metric tonnes ha^{-1}, respectively (Anonymous 2017).

Guava fruit becomes fully ripe between three to five days at room temperature. It is highly perishable, susceptible to mechanical damage and chilling injury and has a limited postharvest shelf life (Ismil *et al.* 2010). It is therefore necessary to reduce rates of these physico-chemical changes and enhance its storage life. for an extended period. The fruit is highly perishable in nature because of high moisture content, thin and soft skin and are subjected to higher rate of transpiration, respiration, ripening and other biological activities, even after harvest, which deteriorate the quality of the fruits in a short period and finally make them unmarketable.

After the fruit have been harvested at optimum maturity, the important post-harvest need is the retention of quality for a longer period so that it can be marketed. The post-harvest losses can be minimized by extension of shelf life through checking the rate of transpiration and respiration, microbial infection and protecting membranes from disorganization (Bisen and Pandey, 2008). During storage, physico-chemical and biological changes affect the final texture and quality of fruits. Post-harvest dipping treatment increases the shelf life of the fruits by retaining their firmness and control of the decaying organism (Ahmad *et al.,* 2005).

Edible coatings are used for extension of shelf life of fruits and vegetables. These can also be safely eaten as part of the product and do not add unfavorable properties to the foodstuff. Edible coatings or films increase the shelf life of fruits and vegetables and are environment friendly. In recent years, new edible films and coatings have been developed with the addition of various and edible herbs, antimicrobial compounds to preserve fresh fruits and vegetables. Edible coatings prevent loss of firmness and moisture (Raghav *et al.* 2012).

Scanty research on this aspect has so far been carried out in M.P. and there is little information available on prolonged shelf life of guava by post-harvest dip treatment. Therefore, the study to assess the effect of starch and oil based edible coatings on shelf life of guava was conducted.

MATERIAL AND METHODS

Fresh, fully mature, uniform sized healthy guava fruits harvested at the colour break stage from ten year old guava trees (*Psidium guajava* L.) cv. Lucknow-49 were used in the study at Fruit Research Station Imaliya, Department of Horticulture, JNKVV, Jabalpur (M.P.). The solutions of corn starch and different oils along with beeswax at different concentrations were prepared in the Department of Horticulture, J.N.K.V.V., Jabalpur. The experiment consisted of 16 treatments *viz.,* corn starch (0%, 2%, 4%, 6%) with oil [sunflower (1%), rice bran (1%), safflower (1%) + beeswax (1%)] and control. Post-harvest dipping of guava fruits was done in December 2019.

Three concentration of cornstarch (2, 4 and 6%) solution was tested. This was prepared by dissolving 20 gm, 40 gm

and 60 gm corn starch in 1 litre distilled water, respectively. For preparation of (1%) sunflower oil + beeswax (1%), (1%) rice bran + beeswax (1%) and 1 per cent safflower oil + beeswax (1%) solution, 10 g of each oil (sunflower, rice bran and safflower) + 10 g of beeswax were combined with 0, 2, 4, 6 per cent corn starch solution.

The fruits after harvesting were washed under running tap water and air dried. Initial parameters were recorded before dipping in the solutions. After that the fruits were dipped in the solution as per treatments and stored at ambient room temperature. The treated fruits were subjected to various physico-chemical observations as per details given below at 3rd, 6th, 9th and 12th day of storage.

Observations on physico-chemical characters of guava fruits with different treatments application were recorded as per the methods given under different characters: Observations were recorded at 0 (initial), 3, 6, 9 and 12 days intervals.

Physiological loss in weight was taken after each storage interval and loss in weight during storage was expressed as per cent of initial weight. The data on fruit decay due to fungus or any micro-organisms infection was recorded every third day and calculated as a percentage of the total number of fruit decayed. The development in surface colour was measured by using Hunter Lab Colorimeter (Hunter Associates Laboratory, Inc., USA) with CIELab scale (L*, a*, and b*).

RESULTS AND DISCUSSION

Physiological loss in weight (%)

Physiological loss in weight during storage is characterized by reduction in fruit weight due to loss of moisture through evaporation and/or transpiration. It is the most important parameter because it governs the post-harvest quality of the guava fruits. Loss in fruit weight drastically reduce the quality of product. It is evident from the present study that loss in weight was significantly affected by different post-harvest treatment throughout the storage period up to 12th day. The fruit coated with sunflower oil (1%) + beeswax (1%) maintained the loss in weight (13.55%) at 12th day of storage (Table 1), whereas maximum loss in weight was observed in control (water) 16.59% during the same period of storage.

The concentration of corn starch significantly minimized the loss in weight,. during 3 to 12 days of storage period. The minimum loss in weight was observed when guava fruits were treated with (2%) corn starch i.e. 4.38 to 14.39 per cent whereas maximum loss of 5.13 to 16.13 per cent was observed under 0% corn scratch (water).

In general, the physiological loss in weight increases with the advancement of storage period. In the present investigation, the minimum physiological loss in weight (12.49%) at 12th day during storage was recorded with T7 (corn starch (2%)+ safflower oil (1%) + 1.0 per cent beeswax) closely followed by T6 (corn starch (2%)+ rice bran oil (1%) +

Table 1: Effect of oil and starch based edible coating on the percentage loss in weight (PLW %) of guava cv. Lucknow-49 during storage

Treatment		PLW%				
T 0	Control	0	7.43	11.13	15.00	20.99
T1	Cornstarch 0% + sunflower oil (1%) + beeswax (1%)	0	4.50	7.81	11.09	15.10
T 2	Cornstarch 0% + rice bran oil (1%) + beeswax (1%)	0	4.62	7.43	11.58	15.49
T 3	Cornstarch 0% + safflower oil (1%) + beeswax (1%)	0	4.48	8.11	10.38	14.79
T 4	Corn starch (2%) + 0 + 0	0	4.14	7.07	10.17	14.26
T 5	Cornstarch (2%) + sunflower oil (1%) + beeswax (1%)	0	3.96	7.01	9.64	13.74
T 6	Cornstarch (2%) + rice bran oil (1%) + beeswax (1%)	0	3.64	6.76	9.57	13.72
T 7	Cornstarch (2%) + safflower oil (1%) + beeswax (1%)	0	3.50	6.69	9.21	12.49
T 8	Cornstarch (4%) + 0 + 0	0	4.55	7.16	10.22	14.04
T 9	Corn starch (4%) + sunflower oil (!%) + beeswax (1%)	0	4.48	7.19	0.28	14.26
T 10	Corn starch (4%) + rice bran oil (1%) + beeswax (1%)	0	4.66	8.11	1.01	14.64
T 11	Corn starch (4%) + safflower oil (1%) + beeswax (1%)	0	4.63	8.18	12.0	15.71
T 12	Corn starch (6%) + 0% + 0%	0	4.39	8.13	11.37	15.23
T 13	Corn starch (6%) + sunflower (1%) + beeswax (1%)	0	4.59	7.63	10.51	14.46
T 14	Cornstarch (6%) + rice bran oil (1%) + beeswax (1%)	0	4.70	7.77	10.83	14.16
T15	Corn starch (6%) + safflower oil (1%) + beeswax (1%)	0	4.82	7.91	11.03	15.05
SE(m) ±		**0**	**0.12**	**0.061**	**0.023**	**0.037**
C.D. at 5% level		**0**	**0.373**	**0.177**	**0.066**	**0.108**

1.0 per cent beeswax) against the maximum (20.99%) physiological loss in weight under T0 (control). The possible reason for reduced weight loss by chemical might be due to evaporation and transpiration processes. The reduction in weight loss was probably due to the effects of these coatings as a semi permeable barrier against oxygen, carbon dioxide, moisture and solute movement, thereby reducing respiration, water loss and oxidation reaction rates (Baldwin *et al*., 1999). Fruits coated with corn starch showed lesser weight loss during storage as compared to fruits under control. (Oluwaseun *et al.* 2013) observed that corn starch coated cucumber showed a significant delay in weight loss compared to uncoated ones. This result is in agreement with the behavior of stand-alone formed films, where an increase in rice bran oil decreased the WVP of the films (Hassani *et al*. 2019).

Fruit size length and diameter (cm)

The size of the fruit (length and diameter) decreased during storage period. However, the treated fruits maintained higher values of fruit size as compared to control. The guava fruit length and diameter decreased with the increase in storage period. The table 2 revealed that the fruit length and diameter was maximum (5.38 and 6.06, respectively) under treatment sunflower oil (1%) + beeswax (1%) treatment, whereas minimum length of fruits (5.07 and 5.71, respectively) was observed under control (water) on the 12th day of storage. Corn starch (2%) was found to have a minimum decrease in fruits length and diameter (5.28 and 5.91). Maximum decrease in fruit length (5.06 and 5.79) occurred in (0% corn starch) i.e. (water).

As for the interaction effect of oil and corn starch, the minimum reduction in fruit length (5.53 cm) and diameter (6.20 cm) was observed in T_7 (corn starch (2%)+ safflower oil (1%)+ beeswax (1%)) and maximum reduction in fruit length (4.47) and diameter (5.61) in T0 (control). The reduction in fruit size during storage period might be due to shrinking of

Table 2: Effect of oil and starch based edible coating on the fruit decay percentage of guava (cv. Lucknow-49) during storage

Treatments	**Fruit decay percentage (%)**				
Oil	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**
T 0 Control	0	11.11	55.55	66.66	88.88
T1 Corn starch 0% + sunflower oil (1%) + beeswax (1%)	0	0	33.33	55.55	77.77
T 2 Corn starch 0% + rice bran oil (1%) + beeswax (1%)	0	0	33.33	55.55	77.77
T 3 Cornstarch 0%+ safflower oil (1%) + beeswax (1%)	0	0	33.33	55.55	66.66
T 4 Cornstarch (2%) + 0 + 0	0	0	33.33	55.55	77.77
T 5 Cornstarch (2%) + sunflower oil (1%) + beeswax (1%)	0	0	22.22	44.44	55.55
T 6 Cornstarch (2%) + rice bran oil (1%) + beeswax (1%)	0	0	11.11	33.33	44.44
T 7 Cornstarch (2%) + safflower oil (1%) + beeswax (1%)	0	0	0.00	22.22	44.44
T 8 Cornstarch (4%) + 0 + 0	0	0	33.33	55.55	66.66
T 9 Cornstarch (4%) + sunflower oil (!%) + beeswax (1%)	0	0	28.44	55.44	77.77
T 10 Cornstarch (4%) + rice bran oil (1%) + beeswax (1%)	0	0	33.33	55.55	66.66
T 11 Cornstarch (4%) + safflower oil (1%) + beeswax (1%)	0	0	33.33	55.55	77.77
T 12 Cornstarch (6%) + 0% + 0%	0	0	44.44	55.55	77.77
T 13 Cornstarch (6%) + sunflower (1%) + beeswax (1%)	0	0	33.33	55.55	77.77
T 14 Cornstarch (6%) + rice bran oil (1%) + beeswax (1%)	0	0	33.33	55.55	66.66
T15 Cornstarch (6%) + safflower oil (1%) + beeswax (1%)	0	0	33.33	55.55	66.66
SE(m) ±	**0**	**2.778**	**5.733**	**0.215**	**8.457**
C.D. at 5% level	**0**	**N\A**	**N\A**	**N\A**	**N\A**

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00015.1

fruits caused by transpiration. It might be due to the anti-senescent action of coatings which had an inhibitory effect on ethylene biosynthesis and retard the activity of enzymes responsible for ripening, cell degradation was prevented which in turn facilitated reduced moisture loss and lesser respiratory gas exchange, hence delay in senescence and lower the shrinkage percentage (Sudha *et al*., 2007).

Fruit volume (ml)

The fruit volume decreased with the advancement of storage period up to 12th day of storage. However, the reduction in fruit volume of guava fruits was significantly affected by various post-harvest treatments. From the table 3, it is observed that the volume remains maximum (107.16) under treatment (sunflower oil (1%) + beeswax (1%), whereas minimum volume (101.33) was observed under control (water) on the 12th day of storage. The result was supported by Kaur *et al.* (2014), Kumar *et al.* (2014) and Malik *et al.* (2015).

Fruit decay (%)

The losses due to decaying of fruit were observed from 6th day onwards and increased later (Table 4). The fruit decaying was significantly influenced by various post-harvest treatments. The decay (%) was minimum (55.55) under treatment [sunflower oil (1%) + Beeswax (1%)], whereas maximum decay (77.77) was observed under control (water) at the 12th day of storage. However, the result showed minimum decayed fruits (22.22 and 44.44%) at 9 and 12 day of storage were obtained under T7 treatments [(Corn starch (2%) + safflower oil (1%) + beeswax (1%)] than the others. Rotting caused due to infection, makes the fruit soft and affected fruits develop bad odour. It was because the experimental group's packaging materials could reduce water loss to a certain extent to avoid strawberry oxidation and reduce respiration, reducing the chance of fungal infection and decaying Wang *et al.* (2019). Martinez-Romero (2006) in coated cherry and Wijewardane *et al.* (2009) in corn starch coated apple.

Table 3: Effect of oil and starch based edible coating on the firmness (Kg/cm²) of guava cv. Lucknow-49 during storage

Treatments	Firmness (Kg/cm²)				
Oil	**0 Day**	**Day 3**	**Day 6**	**Day 9**	**Day12**
T 0 Control	5.73	5.10	4.46	3.76	2.79
T1 Corn starch 0% + sunflower oil (1%) + beeswax (1%)	5.76	5.50	4.83	4.33	3.53
T 2 Corn starch 0% + rice bran oil (1%)+beeswax (1%)	5.73	5.46	4.73	4.33	3.50
T 3 Corn starch 0% + safflower oil (1%) + beeswax (1%)	5.53	5.30	4.76	4.23	3.60
T 4 Corn starch (2%) + 0 + 0	5.70	5.40	4.73	4.36	3.53
T 5 Cornstarch (2%) + sunflower oil (1%) + beeswax (1%)	5.66	5.40	5.03	4.56	3.70
T 6 Corn starch (2%) + rice bran oil (1%) + beeswax (1%)	5.70	5.60	5.26	4.66	3.76
T 7 Corn starch (2%) + safflower oil (1%)+ beeswax (1%)	5.60	5.56	5.36	4.80	4.20
T 8 Corn starch (4%) + 0 + 0	5.76	5.43	4.70	4.23	3.36
T 9 Corn starch (4%) + sunflower oil (!%) + beeswax (1%)	5.66	5.36	4.80	4.40	3.60
T 10 Corn starch (4%) + rice bran oil (1%) + beeswax (1%)	5.80	5.36	4.66	4.26	3.36
T 11 Corn starch (4%) + safflower oil (1%) + beeswax (1%)	5.80	5.37	4.73	4.30	3.40
T 12 Corn starch (6%)+ 0% + 0%	5.73	5.36	4.86	4.20	3.50
T 13 Corn starch (6%) + sunflower (1%) + beeswax (1%)	5.70	5.33	4.83	4.30	3.36
T 14 Cornstarch(6%)+ rice bran oil (1%) + beeswax (1%)	5.60	5.30	4.76	4.43	3.50
T15 Corn starch (6%) + safflower oil (1%) + beeswax (1%)	5.63	5.30	4.83	4.33	3.53
SE(m) ±	**0.072**	**0.039**	**0.023**	**0.065**	**0.044**
C.D. at 5% level	**N\A**	**0.114**	**0.067**	**0.188**	**0.129**

Firmness (Kg/cm²)

Firmness was significantly influenced by various post-harvest treatments. It was found to have minimum reduction (3.70) under treatment [sunflower oil (1%) + Beeswax (1%)], whereas maximum loss in firmness (3.44) was observed under control (water) at 12th day of storage (From table 5). The maximum coatings of rice bran reduces the texture loss up to 5% with respect to the control samples depending on the rice bran oil contents Hassani *et al*. (2012).

Surface colour development

Interaction of oil and corn starch had a significant effect on surface colour development of guava fruits. Maximum colour L,a,b (32.30, 5.15, 23.50) and minimum (23.00, 3.10, 18.40) were recorded under (control) and T 7 (Cornstarch (2%) + safflower oil (1%) + beeswax (1%)) respectively after 12 days of storage. The result was supported by Vishwasrao, and Ananthanarayan 2016).

Table 4: Effect of oil and starch based edible coating on the surface colour development of guava cv. Lucknow-49 during storage

Treatments	Surface colour development														
	Day 0			Day 3			Day 6			Day 9			Day12		
Oil	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
0 Control	25.00	0.40	19.90	26.12	1.90	20.50	27.00	2.80	21.21	31.00	4.60	22.45	32.30	5.15	23.50
T1 Corn starch 0% + sunflower oil (1%) + beeswax (1%)	22.12	0.30	16.15	23.12	1.40	18.15	24.38	2.00	19.10	25.20	3.15	19.90	26.40	4.10	20.00
T 2 Corn starch 0% + rice bran oil (1%) + beeswax (1%)	20.16	0.27	14.10	21.15	1.00	15.00	21.12	1.50	16.15	22.68	2.10	17.15	23.40	3.50	19.12
T 3 Corn starch 0% + safflower oil (1%) + beeswax (1%)	20.14	0.29	14.16	21.13	1.01	15.02	21.15	1.70	16.10	22.70	2.17	17.19	23.34	3.43	19.10
T 4 Corn starch (2%)+ 0 + 0	20.20	0.34	14.11	21.00	1.05	15.05	21.20	1.80	16.16	22.58	2.18	17.34	23.46	3.50	19.17
T 5 Cornstarch (2%)+sunflower oil (1%)+beeswax (1%)	20.14	0.40	14.15	21.25	1.08	15.06	21.17	1.90	16.18	22.60	2.20	17.59	23.40	3.90	19.50
T 6 Corn starch (2%) + rice bran oil (1%) + beeswax (1%)	20.05	0.20	14.10	21.20	1.02	15.01	21.10	1.40	16.12	22.40	2.12	17.40	23.22	3.40	19.30T
T 7 Corn starch (2%) + safflower oil (1%) + beeswax (1%)	20.00	0.12	14.05	21.12	1.01	14.89	21.09	1.35	16.23	22.10	2.03	17.20	23.00	3.10	18.40
T 8 Corn starch (4%) + 0 + 0	21.23	0.20	14.30	21.70	1.40	15.60	21.40	1.80	17.01	22.27	2.40	17.58	23.45	3.40	19.00
T 9 Corn starch (4%) + sunflower oil (!%) + beeswax (1%)	21.26	0.24	14.31	21.76	1.42	15.70	21.42	1.83	17.13	22.30	2.50	17.60	22.50	3.40	19.20
T 10 Corn starch (4%) + rice bran oil (1%) + beeswax (1%)	21.30	0.25	14.30	21.68	1.48	15.72	21.40	1.90	17.18	22.35	2.70	17.75	22.55	3.90	20
T 11 Corn starch (4%) + safflower oil (1%) + beeswax (1%)	21.32	0.28	14.32	21.64	1.43	15.71	21.41	1.90	17.20	22.3	2.71	17.70	22.5	3.92	20.00
T 12 Corn starch (6%) + 0% + 0%	22.00	0.25	14.30	21.60	1.54	15.73	21.40	1.89	17.21	22.30	2.72	17.50	22.60	3.873	20.10
T 13 Corn starch (6%) + sunflower (1%) + beeswax (1%)	22.10	0.30	14.31	21.54	1.48	15.73	21.39	1.87	17.20	22.50	2.78	17.47	22.65	3.87	20.12
T 14 Cornstarch (6%) + rice bran oil (1%) + beeswax (1%)	22.11	0.24	14.33	21.60	1.45	15.65	21.40	1.84	17.12	22.20	2.80	17.58	22.65	3.80	20.12
T15 Corn starch (6%) + safflower oil (1%) + beeswax (1%)	22.10	0.28	14.31	21.63	1.46	15.79	21.43	1.96	17,22	22.21	2.72	17.68	22.67	3.78	20.16

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00015.1

Table 5: Market value of guava fruits after improving shelf life and quality

Name of treatments	Cost of 1 litre of solution (Rs)	Cost of guava fruits(20kg) at the rate of Rs 40 kg^{-1} at 0 day (fresh) (Rs)	Decay fruits after 12th day (kg)	Marketable fruits after 12th day	Market value of the fruits (Rs. kg^{-1})
T0 Water (control)	----	45.00	88.88	1	Rs. 21
T1 Corn starch 0% + sunflower oil (1%) + beeswax (1%)	9.4	45.00	77.77	2	Rs. 30
T2 Corn starch 0% + rice bran oil (1%) + beeswax (1%)	9.34	45.00	77.77	2	Rs. 32
T3 Corn starch 0% + safflower oil (1%) + beeswax (1%)	10.95	45.00	66.66	3	Rs. 32
T4 Corn starch (2%) + 0 + 0	3.5	45.00	77.77	2	Rs. 35
T5 Cornstarch (2%) + sunflower oil (1%) + beeswax (1%)	12.9	45.00	55.55	4	Rs. 35
T6 Corn starch (2%) + rice bran oil (1%) + beeswax (1%)	12.84	45.00	44.44	5	Rs. 40
T7 Corn starch (2%) + safflower oil (1%) + beeswax (1%)	14.45	45.00	44.44	5	Rs. 45
T8 Corn starch (4%) + 0 + 0	7.00	45.00	66.66	3	Rs. 32
T9 Corn starch (4%) + sunflower oil (!%) + beeswax (1%)	16.40	45.00	77.77	2	Rs. 30
T10 Corn starch (4%) + rice bran oil (1%) + beeswax (1%)	16.34	45.00	66.66	3	Rs 32
T11 Corn starch (4%) + safflower oil (1%) + beeswax (1%)	18.8	45.00	77.77	2	Rs 30
T12 Corn starch (6%) + 0% + 0%	10.5	45.00	77.77	2	Rs. 30
T13 Corn starch (6%) + sunflower (1%)+ beeswax (1%)	19.9	45.00	77.77	2	Rs. 30
T14 Cornstarch (6%) + rice bran oil (1%) + beeswax (1%)	19.84	45.00	66.66	3	Rs. 32
T15 Corn starch (6%) + safflower oil (1%) + beeswax (1%)	21.5	45.00	66.66	3	Rs. 32

Market acceptability

The techno- economic feasibility of edible coating of guava was determined by calculating the total cost of treatment of coating of guava. The estimation of cost of treatment was done by using standard calculation method. By the considering of the raw material cost, processing cost for 1 kg of guava the maximum economical return worth ₹ 45 per kg of fruit was obtained with T7, whereas the minimum return worth ₹ 21 per kg of fruit in control (T0) treatment on 12th day of storage

CONCLUSION

It is concluded that the application of combinations of starch and oil based edible coatings may extend shelf life of guava fruits. Coating of guava fruits with corn starch (2%) + safflower oil (1%) + beeswax (1%) proved the best registering minimum loss in physiological weight (12.490%) and fruit decay (44.44%) and maximum fruit size {length- (5.537cm) and diameter- (6.200cm)}, fruit volume (110.66ml) specific gravity (0.877g/ml) and firmness (3.833 kg/cm^2).

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Online Manuscript No. 97711 recived on 18.4.2022
Manuscript accepted for publication on 15.11.2022

Journal of Eco-friendly Agriculture **18**(1): 93-101. 2023
doi: 10.5958/2582-2683.2023.00016.3

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Efficacy of IBA treatments on field performance of terminal and semi hardwood cuttings of crossandra (*Crossandra infundibuliformis*) var. Arka Shravya

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ABSTRACT

The present investigation carried out to assess the field performance of crossandra flower var. Arka Shravya propagated through various cutting types (terminal and semi hardwood) and undole-3-butyric acid (IBA) treatments (100, 200, 300 ppm as a prolonged dip for 24h; 1000, 2000, 3000 ppm as a quick dip for 10 sec and 0 ppm as control) showed that the terminal cuttings pretreated with IBA quick dip @ 3000 ppm for 10 sec superior performance in terms of various growth parameters like plant height (76.40 cm), number of primary branches per plant (11.95), plant spread (1202.20 cm^2), leaf area (1433.78 cm^2) and total leaf chlorophyll content (68.62 SPAD units). The treatment also exhibited earlier spike initiation (11.00), first flower opening (17.00), days to 50 percent (19.50) and complete flowering (22.50) in crossandra var. Arka Shravya.

Keywords: Crossandra, terminal cuttings, semi hardwood cuttings, IBA treatments, vegetative growth, flowering

Crossandra (*Crossandra infundibuliformis* L. (Nees)) belonging to the family Acanthaceae, is native to the Arabian Peninsula, tropical Africa, Madagascar, India and Sri Lanka (Brickell, 1996) and is commonly known as fire cracker plant or Kanakambaram or Kanakadhara. It is an evergreen shrub producing profuse flowers on dense sessile spikes throughout the year with an economic life of 3 years. It is one of the popular Indian loose flowers grown commercially in southern states of India like Andhra Pradesh, Karnataka and Tamil Nadu (Singh, 2006). Though the flowers don't have any fragrance, they are very popular because of their attractive colour, light weight and is employed for making garland, venis and gajras along with jasmine flowers to use as floral ornaments (Singh, 2006). The plant can be useful in landscapes as flowerbeds, borders and it also grown as an ornamental pot flower in Sweden, Denmark and Hungary (Ottosen and Christensen, 1986). With all these uses this flower is ruling the Indian flower market with high prices throughout the year which attracts farmers for its cultivation.

Crossandra can be propagated by seed, cuttings and micropropagation among which seed propagation is widely followed for commercial multiplication. However, the recently developed commercial high yielding crossandra varieties being triploids and not setting the seeds pose serious obstacles in its cultivation. Therefore, the only alternative for multiplication in these varieties is through cuttings. Till date, no research has been carried out on the propagation of crossandra through cuttings. So, the present investigation was carried out to propagate crossandra by cuttings and evaluate their subsequent performance in the main field for growth and flowering attributes.

MATERIALS AND METHODS

The present investigation was conducted at College of Horticulture, Dr. Y.S.R Horticultural University, Venkataramannagudem, Andhra Pradesh, India. The experiment was laid out in a factorial randomized block design with fourteen treatments from two factors i.e., cutting types (terminal cuttings and semi hardwood cuttings) and IBA treatments (100, 200, 300 ppm as a prolonged dip for 24h and 1000, 2000, 3000 ppm as a quick dip for 10 sec and 0 ppm as control) and replicated twice.

Treatment combinations

T_1: Terminal cutting + prolonged dip in IBA solution @ 100 ppm for 24 h

T_2: Terminal cutting + prolonged dip in IBA solution @ 200 ppm for 24 h

T_3: Terminal cutting + prolonged dip in IBA solution @ 300 ppm for 24 h

T_4: Terminal cutting + quick dip in IBA solution @ 1000 ppm for 10 sec

T_5 : Terminal cutting + quick dip in IBA solution @ 2000 ppm for 10 sec

T_6 : Terminal cutting + quick dip in IBA solution @ 3000 ppm for 10 sec

T_7 : Terminal cutting + No IBA treatment

T_8 : Semi hardwood cutting + prolonged dip in IBA solution @ 100 ppm for 24 h

T_9 : Semi hardwood cutting + prolonged dip in IBA solution @ 200 ppm for 24 h

T_{10} : Semi hardwood cutting + prolonged dip in IBA solution @ 300 ppm for 24 h

T_{11} : Semi hardwood cutting + quick dip in IBA solution @ 1000 ppm for 10 sec

T_{12} : Semi hardwood cutting + quick dip in IBA solution @ 2000 ppm for 10 sec

T_{13} : Semi hardwood cutting + quick dip in IBA solution @ 3000 ppm for 10 sec

T_{14} : Semi hardwood cutting + No IBA treatment

Terminal cuttings were taken from top portion of the shoot and semi hardwood cuttings from one-year old shoots of crossandra var. Arka Shravya and pretreated with respective IBA concentrations and planted in polybags containing rooting media (soil, cocopeat, vermicompost and FYM in 2:2:2:1 ratio). Properly rooted, healthy cuttings of 75 days old were transplanted in a well-prepared experimental field at a spacing of 45 cm x 45 cm and 10-15 cm deep. Light irrigation was given immediately after planting. The standard cultural practices were followed throughout the study and five plants were selected at random and tagged in each replication of respective treatments to record observations on vegetative growth and flowering parameters. The results of the experiment were statistically analyzed by the procedure described by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Vegetative growth parameters

Plant height (cm)

Plant height (cm) varied significantly by the influence of various cutting types, IBA treatments and their interactions at 30, 60 and 90 DAP (Table 1) and the mean plant height increased from 38.12 cm (30 DAP) to 64.13 cm (90 DAP). Among two cutting types, terminal cuttings significantly recorded maximum mean plant height of 39.74 cm (30 DAP), 51.12 cm (60 DAP) and 66.48 cm (90 DAP) than semi hardwood cuttings with 36.51 cm, 48.02 cm and 61.78 cm plant height at 30, 60 and 90 DAP. Among IBA treatments, IBA quick dip @ 3000 ppm significantly recorded the maximum mean plant height of 49.67 cm (30 DAP), 62.65 cm (60 DAP) and 75.20 cm (90 DAP) which was significantly followed by IBA quick dip @ 2000 ppm recorded 43.82 cm, 55.85 cm and 71.70 cm at 30, 60 and 90 DAP, respectively, whereas the minimum plant height of 26.50 cm (30 DAP), 36.00 cm (60 DAP) and 41.50 cm (90 DAP) was recorded with IBA @ 0 ppm.

The interaction between cutting types and IBA treatments showed that the maximum plant height (51.65 cm, 64.70 cm and 76.40 cm at 30, 60 and 90 DAP, respectively) was achieved by treating terminal cuttings with IBA quick dip @ 3000 ppm followed by semi hardwood cuttings with IBA quick dip @ 3000 ppm recorded 47.70 cm (30 DAP), 60.60 cm (60 DAP) and 74.00 cm (90 DAP) which was at par with terminal cuttings treated with IBA quick dip @ 2000 ppm (73.40 cm) at 90 DAP. The minimum plant height (27.50 cm and 25.50 cm; 37.00 cm and 35.00 cm; 43.00 cm and 40.00 cm, respectively) was recorded with IBA @ 0 ppm at 30, 60 and 90 DAP in both the types of cuttings viz., terminal and semi hardwood, respectively.

Maximum plant height (cm) was recorded in plants propagated from terminal cuttings which might be due to the early root induction, more number of roots with maximum length was noticed in these plants which helps in more absorption of water and nutrients thus allowing good growth to attain maximum plant height. Cuttings treated with IBA quick dip @ 3000 ppm recorded the maximum plant height because of enhanced enzymatic and photosynthetic activity by the influence of auxins resulting in efficient utilization of photosynthetic products by plants. Auxins induce cell elongation by mounting up the levels of solutes of the cells, by reducing the wall pressure and by increasing the permeability of cells to water which increases the plant height in auxin treated cuttings. The enhanced cell division along with cell enlargement and promotion of protein synthesis is a reason for increased vegetative growth (Evans, 1973) in terminal cuttings treated with high IBA levels (Lal *et al*. 2008 and Girisha *et al*. 2012).

Number of primary branches plant^{-1}

The number of primary branches per plant were found significant by the influence of various cutting types, IBA treatments and their interactions at 30, 60 and 90 DAP. The maximum number of primary branches per plant increased from 3.30 (30 DAP) to 9.45 (90 DAP).

Among cutting types, terminal cuttings recorded maximum mean number of primary branches 3.54, 5.49 and

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00016.3

9.70 per plant at 30, 60 and 90 DAP than semi hardwood cuttings with 3.06 (30 DAP), 5.12 (60 DAP) and 9.20 (90 DAP). The influence of various IBA treatments revealed that IBA quick dip @ 3000 ppm significantly performed the best with mean highest number of primary branches 5.65, 7.30 and 11.67 at 30, 60 and 90 DAP, respectively. This was significantly followed by IBA quick dip @ 2000 ppm produced 4.37 (30 DAP), 6.05 (60 DAP) and 10.75 (90 DAP) primary branches per plant and a minimum i.e., 1.75, 3.40 and 4.47, respectively was revealed at 30, 60 and 90 DAP in treatment of IBA @ 0 ppm.

The number of primary branches per plant varied significantly by the interaction between cutting types and IBA treatments. The highest number of primary branches per plant (6.10, 7.60 and 11.95 at 30, 60 and 90 DAP, respectively) were observed in terminal cuttings treated with IBA quick dip @ 3000 ppm followed by semi hardwood cuttings with IBA quick dip @ 3000 ppm recorded 5.20 (30 DAP), 7.00 (60 DAP) and 11.40 (90 DAP) mean primary branches per plant and the minimum (2.00 and 1.50; 3.80 and 3.00; 4.75 and 4.20) was recorded with IBA @ 0 ppm in both terminal and semi hardwood cuttings at 30, 60 and 90 DAP, respectively.

Maximum number of primary branches were produced in plants propagated through terminal cuttings due to the early establishment of cuttings led to more absorption of water and nutrients thus allowing good growth of plants with the production of more lateral branches. Plants received

Table 1. Plant height (cm) as influenced by cutting types and IBA treatments in crossandra var. Arka Shravya

IBA treatments (G)	**Plant height (cm)**								
	Cutting types (C)								
	30 DAP			60 DAP			90 DAP		
	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean
100 ppm	34.40	32.20	**33.30**	45.10	41.70	**43.40**	64.30	57.70	**61.00**
200 ppm	38.80	33.60	**36.20**	47.20	44.35	**45.77**	67.40	59.50	**63.45**
300 ppm	39.50	35.80	**37.65**	50.70	48.70	**49.70**	70.10	62.50	**66.30**
1000 ppm	41.00	38.50	**39.75**	55.70	51.60	**53.65**	70.80	68.80	**69.80**
2000 ppm	45.35	42.30	**43.82**	57.50	54.20	**55.85**	73.40	70.00	**71.70**
3000 ppm	51.65	47.70	**49.67**	64.70	60.60	**62.65**	76.40	74.00	**75.20**
0 ppm	27.50	25.50	**26.50**	37.00	35.00	**36.00**	43.00	40.00	**41.50**
Mean	**39.74**	**36.51**	**38.12**	**51.12**	**48.02**	**49.57**	**66.48**	**61.78**	**64.13**
Factor	C	G	C x G	C	G	C x G	C	G	C x G
SE (m) ±	0.12	0.24	0.34	0.06	0.12	0.17	0.26	0.50	0.70
CD at 5%	0.39	0.74	1.04	0.20	0.38	0.55	0.82	1.54	2.18

Table 2. Effect of cutting types and IBA treatments on number of primary branches per plant in crossandra var. Arka Shravya

IBA treatments (G)	**Number of primary branches per plant**								
	Cutting types (C)								
	30 DAP			60 DAP			90 DAP		
	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean
100 ppm	2.50	2.50	**2.50**	4.80	4.55	**4.67**	9.65	9.00	**9.32**
200 ppm	2.60	2.50	**2.55**	5.10	4.90	**5.00**	9.90	9.50	**9.70**
300 ppm	3.10	2.70	**2.90**	5.40	5.20	**5.30**	10.20	9.80	**10.00**
1000 ppm	3.60	3.20	**3.40**	5.50	5.40	**5.45**	10.50	10.00	**10.25**
2000 ppm	4.90	3.85	**4.37**	6.25	5.85	**6.05**	11.00	10.50	**10.75**
3000 ppm	6.10	5.20	**5.65**	7.60	7.00	**7.30**	11.95	11.40	**11.67**
0 ppm	2.00	1.50	**1.75**	3.80	3.00	**3.40**	4.75	4.20	**4.47**
Mean	**3.54**	**3.06**	**3.30**	**5.49**	**5.12**	**5.31**	**9.70**	**9.20**	**9.45**
Factor	C	G	C x G	C	G	C x G	C	G	C x G
SE (m) ±	0.009	0.016	0.023	0.005	0.009	0.013	0.002	0.003	0.004
CD at 5%	0.026	0.049	0.070	0.015	0.028	0.040	0.005	0.009	0.013

optimum concentration of auxin i.e., 3000 ppm enhanced vegetative growth by rapid cell multiplication resulted in the promotion of lateral branches. Auxins promote enzymatic activity and photosynthetic activity which was followed by increased food material synthesis and a faster cell division and multiplication resulted in increase in the number of lateral branches (Thimman, 1972). Similar findings were reported by Lal *et al*. (2008) in henna and Ullah *et al*. (2013) in marigold.

Plant spread (cm²)

In the present investigation, both cutting types and IBA treatments significantly improved the mean plant spread from 315.22 cm² at 30 DAP to 858.70 cm² at 90 DAP (Table 3). Terminal cuttings recorded significantly maximum plant spread 329.52 cm² (30 DAP), 564.46 cm² (60 DAP) and 892.08 cm² (90 DAP) followed by semi hardwood cuttings recorded 300.92, 533.55 and 825.33 cm² at 30, 60 and 90 DAP, respectively. All the IBA treatments showed significant differences for plant spread (cm²) throughout the study among which maximum 518.47 cm² (30 DAP), 737.40 cm² (60 DAP) and 1150.55 cm² (90 DAP) was recorded with IBA quick dip @ 3000 ppm followed by IBA quick dip @ 2000 ppm with 405.90 cm², 687.45 cm² and 1061.30 cm², respectively at 30, 60 and 90 DAP. The minimum mean plant spread of 171.25 cm² (30 DAP), 241.75 cm² (60 DAP) and 336.00 cm² (90 DAP) was revealed with IBA @ 0 ppm.

Significant results were observed for interaction between cutting types and IBA treatments for plant spread

Table 3. Effect of cutting types and IBA treatments on plant spread (cm²) in crossandra var. Arka Shravya

IBA treatments (G)	**Plant spread (cm²)**								
	Cutting types (C)								
	30 DAP			60 DAP			90 DAP		
	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean
100 ppm	255.60	246.55	**251.07**	489.35	479.05	**484.20**	679.45	651.55	**665.50**
200 ppm	274.00	244.00	**259.47**	534.65	507.70	**521.17**	912.55	802.20	**857.37**
300 ppm	287.05	253.10	**270.07**	559.35	510.50	**534.92**	935.60	903.15	**919.37**
1000 ppm	351.00	309.60	**330.35**	646.15	626.15	**636.15**	1067.40	974.35	**1020.87**
2000 ppm	411.70	400.10	**405.90**	695.75	679.15	**687.45**	1080.90	1041.70	**1061.30**
3000 ppm	536.75	500.20	**518.47**	750.50	724.30	**737.40**	1202.20	1098.90	**1150.55**
0 ppm	190.50	152.00	**171.25**	275.50	208.00	**241.75**	366.50	305.50	**336.00**
Mean	**329.52**	**300.92**	**315.22**	**564.46**	**533.55**	**549.00**	**892.08**	**825.33**	**858.70**
Factor	C	G	C x G	C	G	C x G	C	G	C x G
SE (m) ±	1.63	3.05	4.31	1.21	2.27	3.21	2.65	4.96	7.01
CD at 5%	5.03	9.42	13.32	3.74	7.00	9.91	8.18	15.31	21.62

Table 4. Leaf area (cm²) as influenced by cutting types and IBA treatments in crossandra var. Arka Shravya

IBA treatments (G)	**Leaf area (cm²)**								
	Cutting types (C)								
	30 DAP			60 DAP			90 DAP		
	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean
100 ppm	153.30	130.68	**141.99**	413.56	378.70	**396.13**	624.19	589.63	**606.91**
200 ppm	190.65	177.82	**184.23**	538.50	507.60	**523.05**	777.72	743.94	**760.83**
300 ppm	218.67	204.86	**211.76**	594.10	555.30	**574.70**	821.56	797.33	**809.44**
1000 ppm	344.76	308.42	**326.59**	674.20	632.10	**653.15**	954.43	869.64	**912.03**
2000 ppm	363.00	346.00	**354.50**	750.30	675.20	**712.75**	1171.07	1043.40	**1107.23**
3000 ppm	452.05	405.89	**428.97**	880.00	832.90	**856.45**	1433.78	1344.07	**1388.92**
0 ppm	125.15	91.30	**108.22**	311.90	278.90	**295.40**	468.70	412.73	**440.71**
Mean	**263.94**	**237.85**	**250.89**	**594.65**	**551.52**	**573.09**	**893.06**	**828.67**	**860.86**
Factor	C	G	C x G	C	G	C x G	C	G	C x G
SE (m) ±	1.47	2.75	3.89	0.50	0.94	1.34	3.00	5.62	7.95
CD at 5%	4.54	8.50	12.02	1.56	2.92	4.13	9.28	17.37	24.56

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00016.3

(cm^2). The maximum plant spread (cm^2) of 536.75 cm^2, 750.50 cm^2 and 1202.20 cm^2 at 30, 60 and 90 DAP, respectively was observed in terminal cuttings treated with IBA quick dip @ 3000 ppm followed by semi hardwood cuttings treated with IBA quick dip @ 3000 ppm recorded 500.20 cm^2 (30 DAP), 724.30 cm^2 (60 DAP) and 1098.90 cm^2 which was on par with (1080.90 cm^2) terminal cuttings treated with IBA quick dip @ 2000 ppm at 90 DAP. The minimum plant spread (cm^2) (190.50 and 152.00 cm^2; 275.50 and 208.00 cm^2; 366.50 and 305.50 cm^2) at 30, 60 and 90 DAP was recorded with IBA @ 0 ppm in both the cuttings viz., terminal and semi hardwood, respectively.

Maximum plant spread was recorded by plants propagated from terminal cuttings which might be due to the early establishment of cuttings, leading to more absorption of water and nutrients thus good growth to allow maximum plant spread. Enhanced metabolic activities of the plants influenced the uptake of water and nutrients due to better establishment of roots in the soil, resulting in increase in plant spread. Another reason may be due to increase in the lateral growth of the plant leading to a higher spread of the plants (Singh, 2003) which was earlier reported by Girisha *et al*. (2012) in daisy.

Leaf area (cm^2)

The influence of cutting types, IBA treatments and their interactions on leaf area (cm^2) depicted in Table 4 showed a significant improvement in mean leaf area from 250.89 cm^2 (30 DAP) to 860.86 cm^2 (90 DAP). Among cutting types, terminal cuttings recorded maximum mean leaf area (cm^2) throughout the study with 263.94 cm^2, 594.65 cm^2 and 893.06 cm^2 at 30, 60 and 90 DAP followed by semi hardwood cuttings recorded 237.85 cm^2 (30 DAP), 551.52 cm^2 (60 DAP) and 828.67 cm^2 (90 DAP). Among IBA treatments, the highest mean leaf area (cm^2) (428.97, 856.45 and 1388.92 at 30, 60 and 90 DAP, respectively) was noticed in IBA quick dip @ 3000 ppm followed by IBA quick dip @ 2000 ppm recorded 354.50 cm^2 (30 DAP), 712.75 cm^2 (60 DAP) and 1107.23 cm^2 (90 DAP), while the lowest mean leaf area 108.22 cm^2, 295.40 cm^2 and 440.71 cm^2 at 30, 60 and 90 DAP was recorded with IBA @ 0 ppm.

The interaction between cutting types and IBA treatments for leaf area was significant and maximum leaf area was observed 452.05 cm^2 (30 DAP), 880.00 cm^2 (60 DAP) and 1433.78 cm^2 (90 DAP) significant in terminal cuttings treated with IBA quick dip @ 3000 ppm followed by semi hardwood cuttings with IBA quick dip @ 3000 ppm recorded 405.89 cm^2 (30 DAP), 832.90 cm^2 (60 DAP) and 1344.07 cm^2 (90 DAP). The minimum leaf area (cm^2) was recorded (125.15 and 91.30 at 30 DAP; 311.90 and 278.90 cm^2 at 60 DAP; 468.70 and 412.73 cm^2 at 90 DAP) with IBA @ 0 ppm in both the types of cuttings viz., terminal and semi hardwood, respectively.

The increased leaf area in terminal cuttings might be due to production of the greater number of leaves with maximum length and width resulted from early establishment of the root system which increased both the activities of photosynthesis and respiration in leaves thus leading to increased number and size of leaves resulting in increased leaf area. Treatment with IBA quick dip @ 3000 ppm recorded highest leaf area due to production of higher number and a maximum length of roots at this concentration and nutrient uptake with healthy and strong root system could have boosted the rate of photosynthesis gaining much stronger position to nurture the growing leaves and expanding them leading to the increase in leaf area (Ismail and Asghar, 2007).

Total leaf chlorophyll content (SPAD)

The total leaf chlorophyll content varied significantly throughout the study by the influence of various cutting types, IBA treatments and interactions and improved the mean leaf chlorophyll content from 50.12 (30 DAP) to 64.95 (90 DAP) (Table 5). A significant increase in mean total leaf chlorophyll content (50.86, 58.54 and 65.63) at 30, 60 and 90 DAP was reported in terminal cuttings followed by semi hardwood cuttings 49.39 (30 DAP), 56.56 (60 DAP) and 64.27 (90 DAP). The influence of various IBA treatments revealed that IBA quick dip @ 3000 ppm significantly performed the best with maximum mean total leaf chlorophyll content 53.55, 62.34 and 68.06 at 30, 60 and 90 DAP, respectively. This was significantly followed by IBA quick dip @ 2000 ppm recorded 51.37 (30 DAP), 60.46 (60 DAP) and 66.76 which was at par with (66.57) IBA quick dip @ 1000 ppm (90 DAP). The minimum mean total leaf chlorophyll content of 46.10 (30 DAP), 53.49 (60 DAP) and 58.00 (90 DAP) was observed in IBA @ 0 ppm.

The interaction between cutting types and IBA treatments for total leaf chlorophyll content was significantly varied and reported maximum in terminal cuttings treated with IBA quick dip @ 3000 ppm recorded 53.94 (30 DAP), 62.52 which was at par with (62.16) semi hardwood cuttings treated with IBA quick dip @ 3000 ppm at 60 DAP and 68.62 (90 DAP). This was significantly followed by semi hardwood cuttings with quick dip in IBA @ 3000 ppm recorded 53.16 (30 DAP), 62.16 which is on par with (61.84) terminal cuttings treated with IBA quick dip @ 2000 ppm at 60 DAP and 67.51 which is on par with (67.31) terminal cuttings treated with IBA quick dip @ 2000 ppm at 90 DAP. The minimum total leaf chlorophyll content (47.54 and 44.66 at 30 DAP; 53.55

which was on par with 53.43 at 60 DAP; 58.98 and 57.03 at 90 DAP) was recorded with IBA @ 0 ppm in both the types of cuttings viz., terminal and semi hardwood, respectively.

Ratnakumari (2014) reported that cuttings with a greater number of leaves enhanced more nutrients uptake by means of increasing the photosynthates production and providing sufficient food material for the metabolic activities of the plants by way of mounting the levels of light harvesting pigments especially chlorophylls as observed in case of terminal cuttings. Growth hormones play an important role in regulating the amount and distribution of assimilates in plants (Galston and Davies, 1969). The increased leaf area with increased concentrations of auxins has activated the process of photosynthesis resulting in more chlorophyll content in leaves (Rani *et al*. 2018).

Flowering attributes

Days to spike initiation

Both the cutting types, IBA treatments and their interactions significantly influenced the number of days to spike initiation in crossandra (Table 6). Earliest spike initiation was reported in terminal cuttings (17.96 days) than semi hardwood cuttings (20.17 days). Quick dip in IBA @ 3000 ppm performed the best with significantly less (12.00) number of days to spike initiation followed by (15.37 days) IBA quick dip @ 2000 ppm and the maximum (27.00 days) delay was reported in control (IBA @ 0 ppm).

Terminal cuttings treated with IBA quick dip @ 3000 ppm has produced the spikes in 11.00 days which was the earliest among all the treatments followed by 13.00 days in

Table 5. Effect of cutting types and IBA treatments on total leaf chlorophyll content (SPAD) in crossandra var. Arka Shravya

IBA treatments (G)	**Total leaf chlorophyll content (SPAD)**								
	Cutting types (C)								
	30 DAP			60 DAP			90 DAP		
	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean
100 ppm	49.97	48.76	**49.36**	57.10	55.11	**56.10**	64.15	63.55	**63.85**
200 ppm	50.29	49.00	**49.65**	57.93	55.15	**56.54**	66.30	64.79	**65.54**
300 ppm	50.96	49.54	**50.25**	58.26	55.28	**56.77**	66.79	64.97	**65.88**
1000 ppm	51.31	49.94	**50.62**	58.60	55.76	**57.18**	67.28	65.86	**66.57**
2000 ppm	52.03	50.70	**51.37**	61.84	59.09	**60.46**	67.31	66.22	**66.76**
3000 ppm	53.94	53.16	**53.55**	62.52	62.16	**62.34**	68.62	67.51	**68.06**
0 ppm	47.54	44.66	**46.10**	53.55	53.43	**53.49**	58.98	57.03	**58.00**
Mean	**50.86**	**49.39**	**50.12**	**58.54**	**56.56**	**57.55**	**65.63**	**64.27**	**64.95**
Factor	C	G	C x G	C	G	C x G	C	G	C x G
SE (m) ±	0.08	0.15	0.21	0.13	0.25	0.35	0.06	0.12	0.17
CD at 5%	0.25	0.47	0.67	0.41	0.78	1.10	0.20	0.37	0.53

Table 6. Effect of cutting types and IBA treatments on days to spike initiation and first flower opening in a spike in crossandra var. Arka Shravya

IBA treatments(G)	**Days to spike initiation**			**Days to first flower opening in a spike**		
	Cutting types (C)			Cutting types (C)		
	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean
100 ppm	21.00	23.50	**22.25**	26.75	29.50	**28.12**
200 ppm	20.00	22.00	**21.00**	26.00	27.75	**26.87**
300 ppm	17.50	21.50	**19.50**	23.25	27.50	**25.37**
1000 ppm	15.50	17.25	**16.37**	21.50	23.00	**22.25**
2000 ppm	14.75	16.00	**15.37**	21.00	22.00	**21.50**
3000 ppm	11.00	13.00	**12.00**	17.00	18.00	**17.50**
0 ppm	26.00	28.00	**27.00**	31.50	33.50	**32.50**
Mean	**17.96**	**20.17**	**19.07**	**23.85**	**25.89**	**24.87**
Factor	C	G	C x G	C	G	C x G
SE (m) ±	0.01	0.02	0.03	0.01	0.02	0.02
CD at 5%	0.03	0.06	0.09	0.04	0.08	0.11

doi: 10.5958/2582-2683.2023.00016.3

semi hardwood cuttings treated with IBA quick dip @ 3000 ppm. Whereas, the maximum number of days to spike initiation (26.00 and 28.00) was reported with IBA @ 0 ppm (control) in both the types of cuttings *viz.,* terminal and semi hardwood, respectively.

The plants propagated from terminal cuttings showed earliness in spike initiation because of early establishment with better synthesis and utilization of photosynthates leading to optimum plant growth would have advanced the flowering in terminal cuttings. The optimum concentration of auxin increases the growth rate and development of plants by rapid cell division and cell multiplication. Distribution of auxin at the periphery of the inflorescence meristems specifies the site of floral meristem initiation in flowering plants. Auxins also play a major role in the development of reproductive organs by specifying the number and identity of floral organs. An increase in the endogenous level of auxin by virtue of its flower bud stimulating characteristics led to earliness in spike initiation in crossandra. Another reason may be enhanced enzymatic activity resulting from the faster mobilization of photosynthates leading to an early transformation from vegetative to reproductive phase (Zimmerman and Wilcoxon, 1935).

Days to first flower opening in a spike

Perusal of data presented (Table 6) reveals that, days to first flower opening in a spike was significantly influenced by the interaction between cutting types, IBA treatments and reported the earliest flower opening (23.85 days) in terminal cuttings than semi hardwood cuttings (25.89 days). Among IBA treatments, IBA quick dip @ 3000 ppm showed earliness in first flower opening in a spike (17.50 days) followed by (21.50 days) IBA quick dip @ 2000 ppm and maximum number of days taken to flower opening in a spike (32.50 days) was noticed with IBA @ 0 ppm (control).

The interaction between cutting types and IBA treatments was significant for days to flower opening in a spike and minimum days to flower opening in a spike (17.00 days) was achieved by treating terminal cuttings with IBA quick dip @ 3000 ppm followed by (18.00 days) semi hardwood cuttings treated with IBA quick dip @ 2000 ppm, whereas maximum delay for flower opening (31.50 and 33.50 days) was reported with IBA @ 0 ppm in both the types of cuttings *viz.,* terminal and semi hardwood respectively.

Early flowering is a qualitative genetic trait regulated by both environmental and endogenous factors. In our study, early flower opening was noticed in plants propagated from terminal cuttings because of early flowering which advanced the flower opening in a spike. Among phytohormones, auxins play an essential role in regulating qualitative traits resulting in advancement in anthesis and early flowering (Grace, 1937). Similar findings were reported by Singh (1999) in hybrid tomato with stem cutting.

Days to 50 percent flowering

Days to 50 percent flowering varied significantly by the influence of various cutting types, IBA treatments and their interactions (Table 7). Terminal cuttings significantly recorded minimum days to 50 percent flowering (26.82) followed by semi hardwood cuttings (29.17 days). Treatment with IBA quick dip @ 3000 ppm showed earliness in days taken to 50 percent flowering (20.75) followed by IBA quick dip @ 2000 ppm (24.37 days) while maximum days taken to 50 pe cent flowering (36.12) was recorded with IBA @ 0 ppm.

The interaction between cutting types and IBA treatments revealed that minimum number of days to 50 percent flowering (19.50) was observed in terminal cuttings

Table 7. Effect of cutting types and IBA treatments on days to 50 percent and complete flowering in crossandra var. Arka Shravya

IBA treatments (G)	Days to 50 percent flowering			Days to complete flowering		
	Cutting types (C)			Cutting types (C)		
	Terminal cutting	Semi hardwood cutting	Mean	Terminal cutting	Semi hardwood cutting	Mean
100 ppm	30.00	32.50	**31.25**	33.50	35.75	**34.62**
200 ppm	28.50	31.00	**29.75**	31.50	34.50	**33.00**
300 ppm	26.50	30.50	**28.50**	29.50	33.75	**31.62**
1000 ppm	24.25	26.25	**25.25**	27.50	30.50	**29.00**
2000 ppm	23.75	25.00	**24.37**	26.75	27.50	**27.12**
3000 ppm	19.50	22.00	**20.75**	22.50	25.00	**23.75**
0 ppm	35.25	37.00	**36.12**	38.00	39.25	**38.62**
Mean	**26.82**	**29.17**	**27.99**	**29.89**	**32.32**	**31.10**
Factor	C	G	C x G	C	G	C x G
SE (m) ±	0.01	0.02	0.02	0.01	0.03	0.04
CD at 5%	0.03	0.06	0.09	0.04	0.09	0.13

doi: 10.5958/2582-2683.2023.00016.3

treated with IBA quick dip @ 3000 ppm followed by (22.00 days) IBA quick dip @ 2000 ppm and maximum delay to 50 percent flowering (35.25 and 37.00 days) was noticed with IBA @ 0 ppm in both the types of cuttings *viz.,* terminal and semi hardwood, respectively.

Early establishment and optimum photosynthate levels with sufficient plant growth was the reason for early flowering in plants propagated from terminal cuttings. Earliness in flower emergence and anthesis by the influence of IBA resulted in the early transformation from vegetative to reproductive phase showed a relation in earliness to 50 percent flowering (Zimmerman and Wilcoxon, 1935). It was found that IBA induced an early outbreak of bud that resulted in early flowering (Pandey and Chandra, 2008).

Days to complete flowering

Days to complete flowering was significantly influenced by various cutting types, IBA treatments and their interactions which were depicted in Table 7. Among cutting types, terminal cuttings recorded significantly minimum number of days to complete flowering (29.89) in a plot than semi hardwood cuttings (32.32 days). Among IBA treatments, IBA quick dip @ 3000 ppm performed the best with minimum number of days to complete flowering (23.75) in a plot followed by IBA quick dip @ 2000 ppm (27.12 days) while the maximum number of days to complete flowering (38.62) was noticed with IBA @ 0 ppm.

Figure 1: Cuttings at nursery stage

Figure 2: Layout of the main field after transplanting

The interaction between cutting types and IBA treatments for days to complete flowering was significant. Minimum number of days to complete flowering (22.50) in a plot was achieved in terminal cuttings treated with IBA quick dip @ 3000 ppm followed by (25.00 days) semi hardwood cuttings treated with IBA quick dip @ 2000 ppm whereas maximum delay to complete flowering in a plot (38.00 and 39.25 days) was noticed in IBA @ 0 ppm in both the types of cuttings viz., terminal and semi hardwood respectively.

The positive effect of advancement in complete flowering in a treatment was explained by the corresponding superiority on earliness in spike initiation, first flower opening in a spike and 50 per cent flowering in that treatment and the effect was attributed to the promotive effect of auxins in terminal cuttings.

Our study revealed that different cutting types and IBA pretreatments significantly influenced the vegetative growth and flowering in crossandra var. Arka Shravya. Use of terminal cuttings and pretreating them with IBA @ 3000 ppm as a quick dip for 10 sec can be recommended for vegetative propagation of crossandra.

CONCLUSION

It was concluded that the terminal cuttings of crossandra penetrated with indole-3-butyrie acid (IBA) quick dip @ 3000 ppm for 10 seconds gave superior performance in terms of various growth and flowering parameters.

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Online Manuscript No. 99410 recived on 5.8.2022
Manuscript accepted for publication on 5.11.2022

Journal of Eco-friendly Agriculture **18**(1): 102-109. 2023
doi: 10.5958/2582-2683.2023.00017.5

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Design and modeling of an automatic colour separating and size grading machine for orange

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ABSTRACT

This research was aimed to develop and model an innovative and simple prototype of a size grading and colour separating machine for citrus fruits. Design feature included three major characteristics: a simple design and ease of use, parts locally available and ease of construction in addition to possibility sizing of most spherical fruits by changing some adjustments. The machine was adjusted to separate Valencia oranges into three colours and grading of the fruits into five categories based on their sizes. In case of colour separator, the fruit was separated with RGB (red, green and blue) colour method by using TCS3200 colour sensor and Arduino Uno Microcontroller to recognize RGB colours of oranges. The fruit was separated by the sensors based on its colour and the actuator, driven by servo motor, separated the citrus fruits based on their colour. The citrus fruit then fell into the place according to the fruit colour. While in the size grader section, the results revealed that the suggested design achieved high sizing efficiency by using the machine at an angle of inclination of the tilted flat (A_i) that corresponded to the coefficient of static friction of fruit types graded. Also, the roller of the grading system must rotate against the fall of the fruit in order not to get stuck between the roller and the tilted flat.

Key words: Separator, RGB, Arduino Uno Microcontroller, sizer

Post-harvest processes for fruits and vegetables affect quality and market value, especially of fruit crops. Post-harvest process for fruits include washing, separating by size, grading, packing, transporting and storage. Separating and grading of fruits increases its quality which is beneficial both to the consumer and the producer. Therefore, any systems which perform these processes will be of great value. Dragan *et al*., (2014) reported that after harvest, fruit crops differ in many properties. Fruit separating performed after harvest is a set of technological operations, whose aim is to separate crops for placement on the market, preservation or consumption as fresh fruit, or industrial processing. In order to put fruit crops on the market, they must be of uniform quality and size. The process of separating by size may play an important role to ensure the high quality of fresh fruits and maturity. Fruit sizing has value in assessing crop health and yield estimation (Zhenglin *et al*., 2018). The fruit gradation is external factor including size, shape, colour, defect, damage, etc. However, consumer choice is always to have fruits with equal size. Proper separating of fruit ensures uniformity in fruit size, reduces packaging and transportation costs and also provides an optimum packaging configuration. Thus, in the packaging industry, grading of fruits based on size is one of the important tasks that are performed. Though human graders can do this task, machine vision has proved to be a great tool that can replace human separators for consistent and reliable judgment in estimating and comparing the size of the fruits. Human graders may make different judgments on the same product at different instances and if done by human graders it will be time-consuming also (Ajay and Amar, 2014). It has been reported that colour and size are the most important features for accurate classification and separating of citrus. Because of the ever-growing need to supply high-quality fruits and vegetable products within a short time, automated grading of agricultural products is getting special priority among many farmer associations. The objective of the study was to design and develop an orange colour separator and size grader machine.

MATERIALS AND METHODS

The working principle of the tool to be made is an orange fruit separator based on colour and size that use servo motors and gravity in case of colour separating and rollers and tilted flat in case of size grading.

Step-1

Colour separating

Citrus fruit was first placed in a container that is above the separator. The power button was pressed to activate the tool, the orange fruit would go down to the selection place. Before the sensor starts reading the colour of the orange fruit, arduino would read the condition of the pause button. If the pause button is in high condition then the entire process will pause and display the number of fruits that have been separated, if the pause button is in low condition then the separating process will run. If the orange fruit that has been read by the sensor is green, the upper servo will direct the fruit to the left (45°) and the fruit will fall into the green container. If the sensor detects citrus fruits greenish yellow, automatically the upper servo will direct the fruit to the right (120°), closing the servo to the left (45°), and the fruit will fall into a greenish yellow container. The oranges in the greenish yellow container and some in the green container would later be exposed to the process of degreening. Ripe oranges often have some green or yellow-green colour in the skin. Ethylene gas was used to turn green skin to orange. Oranges are non-climacteric fruits and cannot ripen internally in response to ethylene gas, though they will de-green externally. When the sensor detects citrus fruits in orange, the upper servo automatically directs the fruits to the right (120°) so that orange citrus fruits will enter the orange container (Fig. 1 and 2).

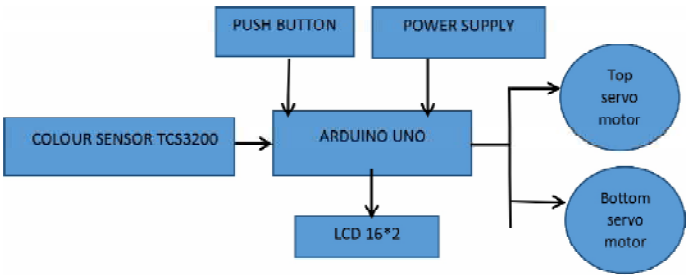

Figure 1: Block diagram of orange fruit separating device

The design consisted of 2 main parts namely the design of hardware systems, and design of software systems. The hardware circuit is shown in Fig. 2.

Layout creation was done by creating a schematic drawing of the circuit using the Proteus 8 ISIS software. Schematic is a series of images that connect components to an electronic circuit.

Step-2

Size grading of the ripened oranges

The grading system consisted of a revolving roller and

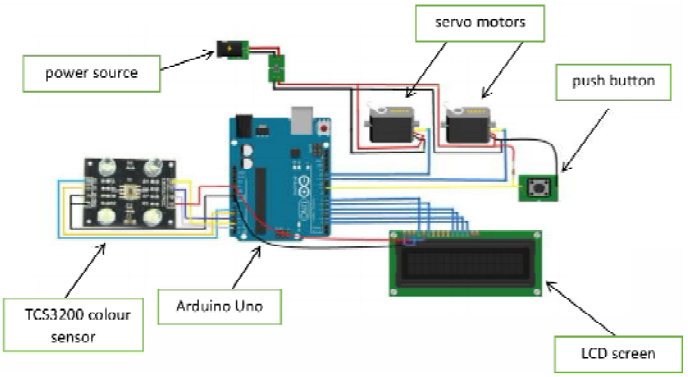

Figure 2: Overall view of orange separator tool

tilted flat. The revolving roller and the tilted flat are the effective parts of the sizing machine. The fruits were graded by controlling the clearance between the revolving roller and the tilted flat. The clearance must adjust according to a type of fruit and the range of fruit sizes before starting the sizing process. In this paper, the sizing machine was adjusted to grade orange fruits into five categories. The size of the fruits graded for the five categories that the machine was supposed to give was calculated by assuming that the fruits are 100 per cent spherical.

Design features of the sizing machine include three major characteristics, *viz.* a simple design and ease of use, consume a very small electric capacity (6 Watt) and parts are from locally available materials. In addition, possibility of sizing most of spherical fruits by changing some adjustments without mechanical damage.

(i) Colour sensor

TCS3200 colour sensor is a sensor that functions to read orange colour and as an input to Arduino Uno. The TCS3200 is a programmable colour sensing module equipped with GY-31 light-to frequency converter that combines 160 cm configurable 8x8 silicon photodiode array as single monolithic CMOS integrated circuit. The output is a square wave (50 percentage duty cycles) with frequency directly proportional to light intensity (irradiance). The full scale output frequency can be scaled by one of the three present values via two control input pins. Digital inputs and digital output allow direct interface to a microcontroller or other logic circuitry. Output enable (OE) places the output in the high impedance state for multiple units sharing of a microcontroller input line. The light-to-frequency converter reads an 8 x 8 array of photodiodes. Sixteen photodiodes have blue filters, 16 photodiodes have green filters, 16 photodiodes have red filters, and 16 photodiodes are clear with no filters. The four types of photodiodes were inter-

digitated to minimize the effect of non-uniformity of incident irradiance. All 16 photodiodes of the same colour were connected in parallel and which type of photodiode the device use during operation was pin-selectable. Photodiodes are 120 mm x 120 mm in size and are on 144-mm center.

The distance between the top surface of the orange and the colour sensor, during the time of detection process, must always be the same. The reason for such a constraint was owed to the fact that TCS3200 produces output signals of different frequencies while detecting the colour of the same object kept at different distances from the sensor.

(ii) Push button

A push-button or simply button is a simple switch mechanism for controlling some aspect of a machine or a process. Buttons were typically made out of hard material, usually plastic or metal. The surface was usually flat or shaped to accommodate the human finger or hand, so as to be easily depressed or pushed. Buttons are most often biased switches, though even many un-biased buttons (due to their physical nature) require a spring to return to their un-pushed state. But in this case push button was a button that functions to temporarily stop all processes that are running and display the total number of fruits that have been separated.

(iii) Arduino Uno microcontroller

Arduino Uno microcontroller provides sets of digital and analog I/O pins that can interface to various expansion boards (termed *shields*) and other circuits. The boards feature serial communication interfaces, including Universal Serial Bus (USB) on some models, for loading programmes from personal computers. For programming the microcontrollers, the Arduino project provides an integrated development environment (IDE) based on a programming language named *Processing*, which also supports the languages C and C++. In this project it was used as a control centre module that can receive input from the sensor and provide an output.

(iv) Servo motors

A servomechanism, sometimes shortened to servo, is an automatic device that uses error-sensing negative feedback to correct the performance of a mechanism and is defined by its function. It usually includes a built-in encoder. A servomechanism is sometimes called a heterostat since it controls a system's behaviour by means of heterostasis.

The term correctly applies only to systems where the feedback or error-correction signals help control mechanical position, speed or other parameters. For example, an automotive power window control is not a servomechanism, as there is no automatic feedback that controls position—the operator does this by observation. By contrast a car's cruise control uses closed loop feedback, which classifies it as a servomechanism. In this machine servo motors are components that function to direct citrus fruits to their respective containers based on their colour.

(v) LCD monitor

A liquid-crystal display (LCD) is a flat-panel display or other electronic visual display that uses the light-modulating properties of liquid crystals. Liquid crystals do not emit light directly. A 16x2 LCD monitor is used to display characters with an amount of 32 characters.

(vi) Fruit sizing system

A fruit sizing system consisted of a revolving roller and tilted flat. The revolving roller and the tilted flat are the effective parts of the sizing machine. The fruits were graded by controlling the clearance between the revolving roller and the tilted flat. The clearance must adjust according to a type of fruit and the range of fruit sizes before starting the sizing process.

RESULTS AND DISCUSSION

Physical and mechanical properties of valencia orange fruits

A summary of the results obtained for the physical and mechanical properties of Valencia orange (Elkaoud and Elglaly, 2019) are shown in Table 1.

Table 1: Physical and mechanical properties of Valencia orange fruits

Properties		Min.	Max.	Mean	Sd	CV %
Axial dimensions, (mm)	L	60	90	74.8	10	13.3
	W	56.7	89.7	71.5	9.3	13.1
	T	53	89.2	70.2	9.2	13
A. diameter, (mm)	Da	56.9	89.6	72.2	9.2	13
Sphericity, (%)	Φ	92.1	99.8	96.5	1.99	2.06
Mass, (g)	M	80	350	190	39.3	20.9
Volume, (cm^3)	V_t	96.5	370.3	207	33.9	16.4
Projected area,	A_P (cm^2)	25.42	63.1	41.6	10.8	25.8
Coefficient of static friction	G. iron	0.24	0.31	0.37	0.05	13.5
	Rubber	0.46	0.69	0.58	0.09	15.5

Axial dimensions

From Table 1, the values of length (L) ranged from 60 to 90 mm with a mean value of 74.8 ± 10 mm, while the values of width (W) ranged from 56.7 to 89.7 mm with a mean value of 71.5 ± 9.3 mm. It was noted that the values of the thickness (T) are not much different from the values of width where it ranged from 53 to 89.2 mm with a mean value of 70.2 ± 9.2 mm. The arithmetic means diameter (Da) was calculated

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00017.5

using the three axial dimensions of fruit, where its value ranged from 56.9 to 89.6 mm with a mean value of 72.2 ± 9.2 mm. There were three possibilities for the fruit to pass through clearance of the fruit sizing system within the developed machine. The fruit falls along its length, width, and thickness.

Sphericity

From Table 1, the value of sphericity ranged from 92.1 to 99.8 per cent and with a mean value of 96.5 ± 1.99 per cent. The sphericity was determined to express the shape of Valencia orange fruits. It has been reported that if sphericity is less than 0.9, the fruit belonged to the oblate group and if sphericity is greater than 1.1, it belonged to the oblong group. The remaining fruits with intermediate index values were considered to be round. Accordingly, Fig. 3 indicates that the frequent per cent (100%) of Valencia orange fruits in the sample was round (sphericity 90–99.8%).

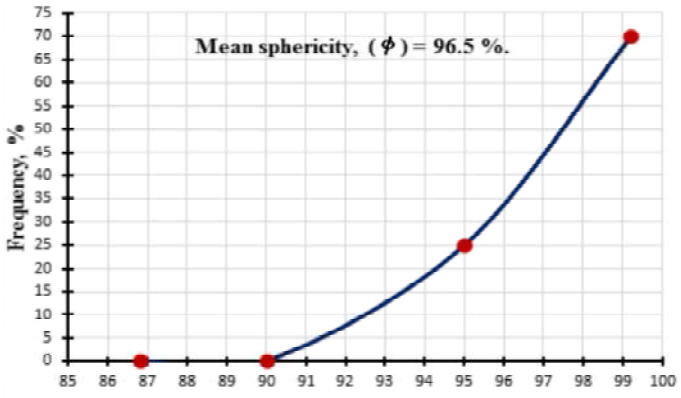

Figure 3: Frequency distribution curve of the sphericity

Mass and true volume

From Table 1, the values of mass and the true volume ranged from 80 to 350 g and from 96.5 to 370.3 cm^3 with mean values of 190 ± 39.3 g and 207 ± 33.9 cm^3 respectively. Fig. 4, shows the relation between mass (g) and the true volume (cm^3) of Valencia orange fruits. This relationship indicated that the correlation between the mass and the true volume is a positive strong correlation (R-squared value = 0.9957) therefore, the resulting equation ($V_t = 0.99 M + 20.2$) could be used to calculate the true volume of Valencia orange fruits by the mass.

Colour separator system test and result

The system tests carried out to determine whether the TCS3200 colour sensor circuit can run properly and can display RGB values on the LCD and serial monitor. The test was done by connecting the colour sensor output pin on the

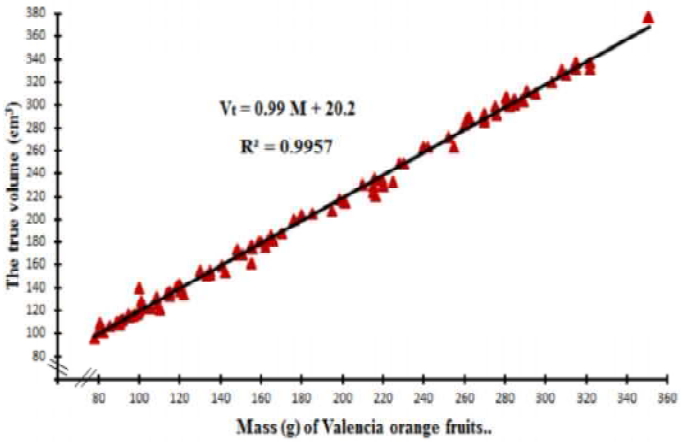

Figure 4: Relation between mass and volume of fruits

Arduino analog pin (A0, A1, A2, A3, A4). In the testing phase this was done using orange fruit that was orange. The citrus fruits were placed just above of the colour sensor to read the RGB (red, green or blue) data values on the LCD and serial monitor. The tests were carried out to determine whether the servo motor circuit can run well and can receive orders from the Arduino to the citrus fruits to the right and left in accordance with the specified container. To apply citrus fruit separating equipment according to this research, which was assembled involved a colour separator that was able to detect yellow (ripe) orange, yellowish green (almost mature) and green (immature) as shown in the Figure 5.

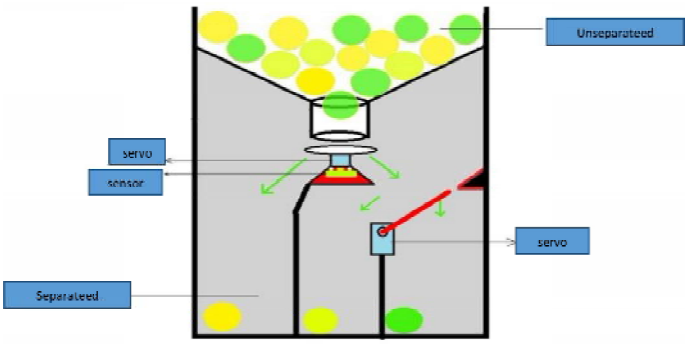

Figure 5: Prototype of orange separating device

Design of fruit hopper

The base area of the prism section ($Area_{b1}$) and the smaller base area of the square pyramid frustum ($Area_{b2}$) were as per equation 3.

$$Area_{b1} = L_1^2$$

$$Area_{b1} = (300)^2$$

$$Area_{b1} = 90{,}000 \text{ mm}^2$$

$Area_{b2} = L_2^2$

$Area_{b2} = (95)^2$

$Area_{b2} = 9025\ mm^2$

From equation 2, the volume of the square pyramid frustum section ($V_{pyramid}$) was:

$$V_{pyramid} = \frac{h_1 B_1 - h_2 B_2}{3}$$

$$V_{pyramid} = \frac{(54.6 \times 90000) - (17.29 \times 9025)}{3}$$

$V_{pyramid} = 1585985.9\ mm^3$

From equation 3, the volume of the prism section (V_{prism}) was:

$h = h_1 - h_2$

$h = 54.6 - 17.29$

$h_3 = h_{total} - h$

$h_3 = 380 - 37.31$

$h_3 = 342.69\ mm$

$V_{prism} = L_1^2 \times h_3$

$V_{prism} = (90000)^2 \times 342.69$

$V_{prism} = 30842100\ mm^3$

From equation 4, the total volume of the fruit hopper (V_{total}) was:

$V_{total} = V_{pyramid} + V_{prism}$

$V_{total} = 1585985.9 + 30842100$

$V_{total} = 32428085.9\ mm^3$

The equations above helped us determine the total internal volume of the hopper which was 32428085.9 mm^3 since the hopper was made of a prism section and pyramid frustum section. And it also helped to determine its capacity by relating it to the volume of the Valencia oranges.

From equation 5, the capacity of the hopper in terms of orange ($N_{oranges}$) was:

$$N_{oranges} = \frac{V_{hopper}}{V_{orange}}$$

Case 1: when the oranges with the smallest dimensions was considered:

$$N_{oranges} = \frac{0.03243}{9.65 \times 10^{-5}}$$

$N_{oranges} \approx 336$ oranges

Case 2: when the oranges with average dimensions was considered:

$$N_{oranges} = \frac{0.03243}{2.07 \times 10^{-4}}$$

$N_{oranges} \approx 157$ oranges

Case 3: when the oranges with the maximum dimensions was considered:

$$N_{oranges} = \frac{0.03243}{3.703 \times 10^{-4}}$$

$N_{oranges} \approx 88$ oranges

From the equations above, we determined that the hopper could have a capacity of a minimum capacity of 88 oranges to a maximum of 336 oranges.

From equation 6, the mass of the oranges in the hopper ($M_{oranges}$) was:

$M_{oranges} = N_{oranges} \times m_{orange}$

Case 1: when the oranges with the smallest mass was considered:

$M_{oranges} = 336 * 80$

$M_{oranges} = 26880\ g$

Case 2: when the oranges with average mass was considered:

$M_{oranges} = 157 * 190$

$M_{oranges} = 29830\ g$

Case 3: when the oranges with the maximum mass was considered:

$M_{oranges} = 88 * 350$

$M_{oranges} = 30800\ g$

The mass of the material used in the fruit hopper when its empty was calculated from equations: 7, 8 and 9.

$V_{pr} = 4 \times L_1 \times h_3 \times t$

$V_{pr} = 4 \times 300 \times 342.69 \times 3$

$V_{pr} = 1233684\ mm^3 = 1.234 \times 10^{-3}\ m^3$

$M_{pr} = \rho_{iron} \times V_{pr}$, where $\rho_{iron} = 7200 kg/m^3$

$M_{pr} = 7200 \times 1.234 \times 10^{-3}$

$M_{pr} = 8.8848\ kg$

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doi: 10.5958/2582-2683.2023.00017.5

$A_{py} = 4\ (109.07 \times 95) + 8\ (\frac{1}{2} 102.5 \times 109.07)$

$A_{py} = 86165.3\ mm^3$

$V_{py} = 86165.3 \times 3$

$V_{py} = 258495.9\ mm^3 = 2.585 \times 10^{-4}\ m^3$

$M_{py} = \rho_{iron} \times V_{py}$

$M_{py} = 7200 \times 2.585 \times 10^{-4}$

$M_{py} = 1.8612\ kg$

$M_{total} = M_{pr} + M_{py} + M_{oranges}$

$M_{total} = 8.8848 + 1.8612 + 30.8$

$M_{total} = 41.5\ kg$

The mass calculated above helped in calculating the frame dimension that safely supported it during operation.

Shaft design considerations

- the load was uniformly distributed
- the shaft was held on beam
- the shaft was held on beam with a bearing
- type of material used (mild steel)
- Yield point of mild steel = 480 N/mm^2
- factor of safety of mild steel was 2

Calculation and discussion of the shaft

From equation 10, the vertical forces (V_f) acting on the shaft length were :

$V_f = T_1\cos\theta + T_2\cos\theta$

$V_f = 45.6\cos(3) + 19.3\cos(3)$

$V_f = 65\ N$

$W_{pulley} = 0.367\ N$

$W_{belt} = 0.23\ N$

Total vertical force = $V_f + W_{pulley} + W_{belt}$

Total vertical force = 65 + 0.367 + 0.23

Total vertical force = 65.6 N

Force analysis of vertical stress on the shaft:

$$\circlearrowright \sum \_\ 0 = -1600 * F_B + 1800 * 65.6$$

$F_B = 74\ N$

(+ve) $\sum \_\ 0 = F\_A + F_B$.

$0 = F_A + 74 - 65.6$

$F_A = -8.4\ N$

Checking the force (F_D) at 900 mm:

$$\circlearrowleft \sum \_\ 0, \quad F\_B \times 200 = F_D \times 900$$

$F_D = 16.5\ N$

The vertical bending moment at A (M_{AV}) and C (M_{cv}):

$M_{AV} = M_{cv} = 0$

Bending moment at D:

$$\circlearrowright M_{DV} = F_{AV} \times 900$$

$F_{AV} = 7560$ N.mm

Bending moment at C:

$$\circlearrowright M_{cV} = F_{BV} \times 200$$

$F_{BV} = 14800$ N.mm

Force analysis of horizontal forces acting on the shaft:

From equation 11, the horizontal forces (H_f) acting on the shaft length were:

$H_f = T_1\sin\theta + T_2\sin\theta$

$H_f = 45.6\sin3 + 19.3\sin3$

$H_f = 3.4\ N$

$$\circlearrowright \sum \_\ 0 = 1600 * F_B + 1800 * 3.4$$

$F_B = 3.8\ N$

(+ve) $\sum \_\ 0 = F\_A + F_B + F_C$

$F_A + 3.8 - 3.4 = 0$

$F_A = -0.4\ N$

Checking the force (F_D) at 900 mm:

$$\circlearrowleft \sum \_\ 0, \quad F\_B \times 200 = F_D \times 900$$

$F_D = 0.75\ N$

The horizontal bending moment at A and C:

$M_{AH} = M_{CH} = 0$

Bending moment at D:

$$\circlearrowleft M_{DH} = F_{AH} \times 900$$

F_{AH} = 360 N.mm

Bending moment at C:

$$\circlearrowleft M_{cH} = F_{BH} \times 200$$

F_{BH} = 680 N.mm

From equation 12, the resultant bending moment acting on the shaft at points D and C were:

$$M_D = \sqrt{M_{DV}^2 + {M_{DH}}^2}$$

M_D=7560^2+360^2

$$M_D = 7568.5\ N.mm$$

$$M_C = \sqrt{M_{CV}^2 + {M_{CH}}^2}$$

M_C=14800^2+680^2

$$M_C = 14815.6\ N.mm$$

The bending moment was maximum at point C:

M = M_c = 14815.6 N.mm

From equation 13, torque exerted on the driving pulley(T) was:

T = (T_1 – T_2) r1

T = (45.6 – 19.3) * 50

T = 1315 N.mm

From equation 14, the equivalent twisting moment (T_e) was:

$$T_e = \sqrt{M^2 + T^2}$$

T_e=14815.6^2+1315^2

$$T_e = 14873.8\ N.mm$$

From equation 15, the equivalent bending moment (M_e) was:

$$M_e = \frac{1}{2}\left[M + \sqrt{M^2 + T^2}\right]$$

M_e=1/214815.6+14873.8

$$M_e = 14844.7\ N.mm$$

From equation 16, the working stress of the material (σ_w) was:

$$\sigma_w = \frac{\sigma_y}{F.O.S}$$

$$\sigma_w = \frac{480}{2}$$

$$\sigma_w = 240\ N/mm^2$$

The permissible shear stress (τ_{max}) was:

τ_{max} = 0.3 * 240

τ_{max} = 72 N/mm^2

The forces, the equivalent bending moment and the equivalent twisting moment that were analysed above helped us determine the correct shaft diameter.

From equation 17, the diameter of the shaft (d) was determined:

$$T_e = \frac{\pi}{16} \times \tau \times d^3$$

$$14873.8 = \frac{\pi}{16} \times 72 \times d^3$$

d = 8.57 mm

The Power Required

Weight of roller with pulley = 6.5 kg

Speed of the roller (ω_r) = 10 rpm

Speed of the roller (v_r) = 0.027 m/s

Length of the fruit sizing system = 125 cm

The calculate power required was as:

Weight of roller with pulleys = 6.5 kg

N. fruits = L/D_a =125/7.2

W_a = 17 * 0.2 = 3.4 kg

Total weight = 6.5 + 3.4 = 9.9 kg

Force = W × μ

So, force = 9.9 * 9.81 * 0.4 = 39

Power required = 39 * 0.027 = 1.1 watt

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00017.5

CONCLUSION

Based on the results of the analysis, design and implementation that have been done above, some conclusions were obtained as: TCS3200 colour sensor could detect the orange fruit colour very well. In this study the colour sensor was very influential on the surrounding light, so it was necessary to do three stages of automatic calibration in the colour reading stage. The separating process started from the fruit colour reading, directing the fruit to the appropriate container and returning to the starting position.

The conclusion on the colour separator and size grader machine was also done. The hopper had a capacity of 32428085.9 mm^3. It could hold 88-336 Valenica oranges. And during operation its total mass could range from 37.626-41.5 kg. The face width of the pulley was 6.25 mm and pulley thickness was 6.5 mm. The diameter of the driver pulley was assumed to be 100 mm and the diameter of the driven pulley was calculated to be 80 mm. The belt had a length and width of 1003 mm and 5 mm, respectively, having a cross sectional area of $2.17 * 10^{-5} m^2$. The coefficient of friction between the belt and the pulley was 0.279. The values of Tension 1 and Tension 2 were 45.6 N and 19.3 N. The power required to drive the shaft was 1.1 watt and a motor of 6 watt was considered to be suitable.

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Manuscript recived on 20.8.2022

Manuscript accepted for publication on 23.9.2022

Journal of Eco-friendly Agriculture **18**(1): 110-114. 2023
doi: 10.5958/2582-2683.2023.00018.7

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Genes expression analysis during fruit development and jelly seed in mango

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ABSTRACT

Mango is an important commercial crop in India that supports livelihood options for orchardists. *Dashehari* mango, grown in northern India is one of the best mango varieties, as it is delicious in taste and aroma and has a high pulp. Dashehari has gained popularity because of its unique taste and ûavour. In the present study expression analysis (RT-PCR) of 14 different genes was analyzed during fruit development and ripening (jelly seed) *i.e.* 30 dpa, 50 dpa, 75 dpa, and jelly seed in mango cv. *Dashehari*. Expression of *ERF119* and *RAP2-12* was found to be high during fruit development and jelly seed pulp suggests *ERF119* and *RAP2-12* play an important role during normal fruit development as well as mango ripening. Expression of *ACO* and *ACS* was found at 75 dpa and ripening. Expression of MYB5 was found during 30 dpa and 50 dpa while CNR, AUX1, PR, peroxidase, and PG were expressed throughout development and ripening. Expression of expansin was down-regulated from 30 dpa, 50 dpa, 75 dpa, and high at jelly seed (ripe). Expression patterns of the genes during fruit development and jelly seed indicated that they are involved in mango fruit development and jelly seed formation by the different transcription factor signalling pathways.

***Key words*:** Fruit development, jelly seed, Ethylene responsive transcription factors

The mango (*Mangifera indica* L.) is one of the major fruit crops of tropical and sub-tropical regions of the world, especially in Asia. Mango belongs to the family Anacardiaceae and is popularly known as the king of fruits. Mango production ranked fifth among the fruit crops worldwide and the second most important tropical fruit crop. It is commercially grown in India, Australia, Nigeria, China, Pakistan, the Philippines, Myanmar, and Egypt. Fruits are valued for their attractive colours, aroma, delightful taste, and high nutritional value (Mukherjee and Litz, 2009). India possesses a large diversity of mango, where an estimated number of cultivars exceeds 1000. Dashehari is one of the leading mango varieties of North India and the area under this variety is increasing in other parts of the country also. The variety is relished due to its pleasant taste, aroma, and high pulp content. In recent years during fruit ripening, jelly seed development has experienced one of the major disorders in some of the pockets, where Dashehari is commercially grown. Fruit ripening is characterized by textural changes, cell expansion and softening by cell-wall solubilization, degradation of starch and chlorophyll, high respiration, and ethylene bust (Ahmad *et al*., 2020).

India possesses a large diversity of mango and the morphological variation among varieties creates complexity in cultivar identification and classification (Riechmann *et al*., 2000). Mango is the most important commercial crop in India which supports livelihood options for orchardists. Mango fruits are an important source of macronutrients such as carbohydrates, lipids and fatty acids, protein, and amino acids as ripe mango contain about 15 per cent of total sugars. The beneficial effects of the mango components on human health due to their dietary, nutritional, and biological properties are affected by fruit development, ripening, and senescence. The maturity stage is a significant aspect that affects the compositional quality of fruit including nutritional factors since during fruit ripening important biochemical, physiological, and structural changes occur (Maria *et al*., 2019). The chemical components of mango vary according to the region of planting, cultivar, cultural practices, and nutritional conditions of the plant. However, the mango fruit development and ripening involve a series of biochemical, physiological, and structural controlled changes that affect the content of nutrients, phytochemical compounds, and organoleptic characteristics leading to a soft, ripe, and edible mango fruit with desirable attributes for consumer acceptance.

The Dashehari cultivar of mango, grown in Northern India is considered one of the best mango varieties, as it is

delicious in taste, and aroma and has a high pulp. However, the consumption of this variety is limited because of its highly perishable nature and has a limited shelf life due to postharvest desiccation and senescence which limits its global distribution. Dashehari has gained popularity because of its unique ûavor but it is tedious for mango growers due to its irregular bearing, cultivation locality-dependent and environment-governed variation in the fruit quality, and physiological disorders like jelly seed (Ahmad *et al*., 2020). Though physiological, biochemical, and few molecular studies have been reported earlier, no serious attempt has so far been made to characterize ethylene-responsive and ripening-related genes during fruit development and the jelly seed of the mango. These genes were selected based on various published data on fruit development and ripening in different fruit crops including mango and our jelly seed transcriptome data. The objective of this study was to characterize the expression of ethylene-responsive and ripening-related genes during fruit development and the jelly seed of the mango.

MATERIALS AND METHODS

Frozen mango pulp samples (30 dpa, 50 dpa, 75 dpa, and jelly seed pulp) were ground to a fine powder with liquid nitrogen using a mortar and pestle. Total RNA was isolated using Spectrum™ plant total RNA kit (Sigma, USA). To remove any traces of genomic DNA total RNA was treated with DNase I (Sigma, USA) according to the protocol. The purity and quantity of total RNA were determined on NanoDrop. For each sample, 2 µg of total RNA was reverse transcribed using the Maxima first strand cDNA synthesis kit for semiquantitative RT-PCR (Genetix) in a 20-µl reaction using Oligo dT and random primers according to the manufacturer instructions. The complementary DNAs (cDNAs) were diluted at 1:10 with nuclease-free water before the RT-PCR analysis. The CDS sequences obtained in jelly seed transcriptome data were used for primer designing using PrimerQuest software with the parameters: optimal length 20-25 base pairs, GC content 50-55 per cent, melting temperature 58-60 °C, amplicon length range 100-250 base pairs and then checked for the absence of stable hairpins and dimers using Oligo Analyzer. Primer sequences for ERF4, RAP2-12, PR, and Peroxidase were taken from other published data. PCR amplification was performed in a total volume of 10 µl by mixing 150 ng of cDNA, 0.5 µm concentration of each primer, 2.5 mM dNTPs and 1 unit of *Taq* DNA polymerase in 1x PCR buffer. The reaction was subjected to initial denaturation of 94 °C for 5.0 min followed by 35 cycles of 94 °C for 30 sec, 58-60 °C for 30 sec, 72 °C for 30 sec with a final extension of 72 °C for 5 min in a thermal cycler (Prima™96, Hi-Media). The PCR amplified products were analyzed by running in 1.5 per cent agarose gel, prepared in 1xTBE buffer, and containing 0.5 µg ethidium bromide and photographed over a transilluminator.

RESULTS AND DISCUSSION

Expression analysis (semiquantitative RT-PCR) of 14 different genes (Table 1) was analyzed at 30 dpa, 50 dpa, 75 dpa, and jelly seed (ripe) mango pulp (Fig. 1). Mango being climacteric fruit its development and ripening is regulated by different phytohormone. Understanding the key genes involved in ethylene biosynthesis and its Ethylene responsive transcription factors (ERFs) is crucial to manipulate their expression for preventing losses due to over-ripening. In this study, SAM synthase (SAM) was expressed throughout development and ripening. While expression of *ACC* oxidase (*ACO*) and *ACC* synthase (*ACS*) was found at 75 dpa and ripening. Climacteric ethylene biosynthesis includes the conversion of amino acid methionine to *S*-adenosylmethionine by SAM synthase. Ethylene is synthesized from SAM by the sequential action of two ethylene biosynthetic enzymes. SAM also has the potential

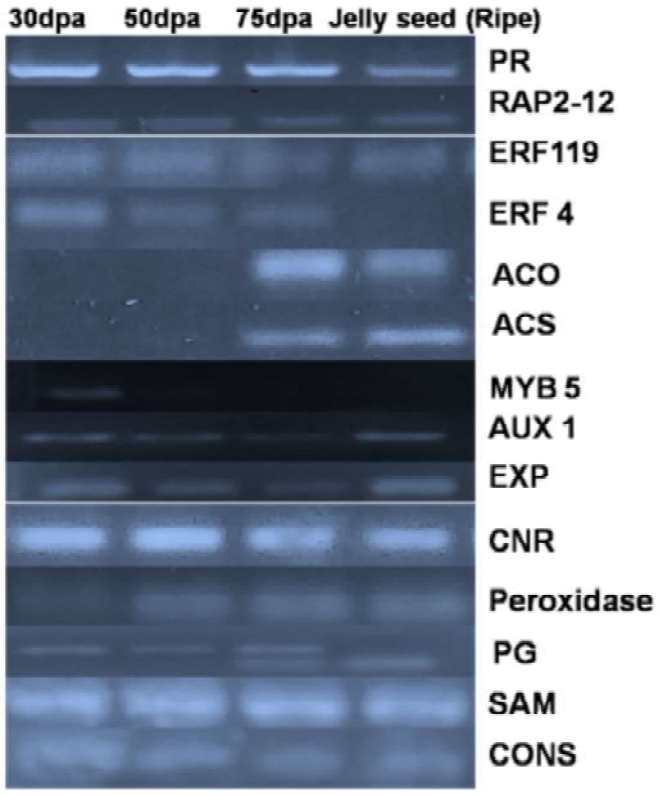

Figure 1: Semiquantative RT-PCR analysis of genes throughout developmental and jelly seed in mango

Table 1. Primers used for semiquantative RT-PCR analysis

Gene	Forward primer (5'-3')	Reverse Primer (5'-3')
CNR	GAACCTTGGACTACTGGGATTT	ATGACAAAGGCAGGGTGTAG
ERF4	AACCCTCCGTCTCCTCTTGT	GCCTCTTCCTGACTCCTCTG
ERF119	TGACCTGGATACTTCTGCTTCA	CTGTTCTTCAAACGGCTGCT
RAP2-12	AAGGTCCAAAGGTTCACAGG	GGAGGGTGCTAATCCAATGA
PR	ATTCTCCTGATTCTCTTTTCGTGC	CCTTGTTGTTGCCTTCCATTG
SAM	CATGGGGACGCTGGGCTTAC	TCCACCTTGGTGGGGTCCTT
AUX1	CCCAACTGGGTATCCTCTCA	AGGCCAACAGCTTTCCAATA
ACO	GTTGGTGACTTGGACTGG	GCCCTAGATTCTCACAGAGC
ACS	GGCATCACCCTAGACAGAGATA	GAACTTGGGTGGACAGAAGAG
CONS	GGCTTCACCAGGAAGGCGAG	CCGGGACAAAAGGAAGGGGG
EXP	GATCCAGGGAGACACCATTTAG	GCTTGTGGCTATGGAAACTTG
Peroxidase	TGCCCAGGAGTTGTCTCTTGT	CAGGTTTGACCTCCATCCAAA
PG	CAGGTCCTTGCAAACCAAATC	ATAGTTCCACCACCTTCCAC
MYB	GCAAGGTAGGGTTAAAGAGAGG	AGACGGCAACTCTTTCCAC

to participate in polyamine or ethylene biosynthesis or both. Although SAM is not exclusively used for ethylene synthesis. Ethylene plays an essential role in the ripening of climacteric fruits. Ethylene-responsive transcription factors regulate the expression of target genes in the ethylene signal transduction pathway by binding to the GCC box in their promoter regions. These target genes in turn regulate the firmness, aroma, taste, colour, and shelf life of the fruits (Nath *et al*., 2006). A number of the *ERF* genes identified in tomato as well as mango are ethylene inducible and show a ripening-related expression pattern that highlights their putative role in fruit ripening (Liu *et al*., 2015; Ahmad *et al*., 2020). In the present study expression of *ERF119* was found high during fruit development and Jelly seed while RAP2-12 expression was high at 30 dpa and 50 dpa but low at 75 dpa and Jelly seed (ripening). Expression of ERF 4 was high at 30 dpa and decreased during development. These data suggest ERFs genes play a very important role during mango fruit development as well as ripening. Specific accumulation of their transcripts in different stages of fruit development indicates their involvement in stage-specific developmental activities (Sharma *et al*., 2010). Mango is a climacteric fruit, there is ethylene production during fruit development and ripening stage. Probably up-regulation of these genes has a major role in climacteric fruit development and ripening. Indeed, climacteric fruits have an absolute requirement for ethylene to ripen and reduction in the hormone synthesis or interference with its perception inhibits this process. Inhibition or delay in fruit ripening by an antisense strategy targeting *ACS* or *ACO* in tomato provided direct evidence that ethylene biosynthesis is essential for climacteric fruit ripening (Picton *et al*., 1993). In this study Low level of auxin-responsive protein (AUX1) is expressed throughout development and ripening. *AUX1* plays a very important role during mango fruit development and ripening. In tomato, a model plant in fruit development studies, many *IAA* genes were identified as being involved in fruit development and ripening (Su *et al*., 2015). In plants, the expression levels of *IAA* family genes were regulated by different hormones. Previous studies have presumed a close relationship between auxin and fruit development and ripening in various plant species (Breitel *et al*., 2016). Endogenous IAA plays role in the flower and fruit development of mango.

Expression of MYB5 was found during 30 dpa and 50 dpa only suggesting MYB 5 does not play a role during maturation and ripening. The MYB has been implicated in many biological processes, such as growth and development, primary and secondary metabolism, especially phenylpropanoid metabolism, stress, and hormonal responses (Arce Rodríguez and Ochoa Alejo, 2017). In pomegranates, the expression of *PgMYB5-like* showed a tissue-based pattern, highest values in flowers and decreasing during fruit development, when anthocyanins increase (Arlotta *et al*., 2020). Transcription factors (TFs) regulate the expression of downstream effector genes, together with ethylene, to coordinate these changes.

The present study revealed high-level expression of pathogen-responsive (PR) and *Colourless non-ripening (*CNR) genes which were found throughout development and ripening. Pathogen-responsive (PR) proteins comprise a group of inducible and functionally diverse proteins that accumulate in response to pathogen attack. These proteins have been implicated in active defense, potentially restricting pathogen development and spread. The PRs have also been reported to be during development, germination, senescence, fruit development, and ripening (Kesari *et al*., 2010). *Colourless non-ripening* encodes a SQUAMOSA promoter-binding protein-like TF. The *Cnr* is completely dominant for ethylene production and pericarp density but incompletely dominant

for fruit pigmentation. The *Cnr* affects fruit size and production of fruit volatiles. The mutation *in Cnr* gene represses normal ripening in a genetically dominant fashion in tomato (Rufang *et al*., 2020). Expression of CNR and PR genes suggests its important role during fruit development and jelly seed (ripening) in mango. Expression of peroxidase was high during development and ripening. Peroxidases maintain peroxy radicals and H_2O_2 at a stable level in plants and are extensively involved in many physiological processes, such as resistance to pathogen infection, lignin formation, auxin decomposition metabolism, seed germination, and aging. In pears expression levels of peroxidase genes were lower at early stages, and gradually increased from the early to the middle stage then decreased during pear fruit ripening. Expression of Polygalacturonase (PG) was high during development and ripening. Expression of expansin was slightly lower at 75 dpa and further increased at the jelly seed (ripening). The PG is the major cell wall degrading enzyme of fruit. It is developmentally regulated and is synthesized *de novo* in ripening tomato fruit. Several PG and expansin genes have been identified in fruits like mango, banana, oil palm, papaya, and cucumber. The PG and expansion proteins disassemble the polysaccharide network of fruit cell walls during ripening and thereby, enable softening. During mango fruit ripening the action of at least these nine PGs contribute to softening (Dautt-Castro *et al*., 2019). Expression of Constain genes (CO) was found throughout development and ripening. In bananas, *constans* gene is expressed highly in the flower and in the fruit pulp suggesting that may be involved in pulp ripening (Chaurasia *et al*., 2016).

In this study, semiquantitative RT-PCR analysis of 14 genes from different functional groups *i.e.* hormonal (ACS, ACO, SAM, ERF, AUX), transcription factors (MYB5), cell wall metabolism (Expansin, PG) oxidative stress (Peroxidase, PR) and others revealed that these genes and transcription factors have developmental and ripening roles. Expression patterns of the genes during fruit development and jelly seed indicated that they are involved in mango fruit development and jelly seed formation by the different transcription factor signalling pathways. Our findings lay a groundwork for further functional characterization of the hormonal and transcription factor family in mango and its role in mango fruit and jelly seed development.

ACKNOWLEDGEMENT

The authors are grateful to the Indian Council of Agricultural Research, New Delhi for financial support.

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Online Manuscript No. 100467 recived on 8.8.2022
Manuscript accepted for publication on 18.11.2022

Journal of Eco-friendly Agriculture **18**(1): 115-118. 2023
doi: 10.5958/2582-2683.2023.00019.9

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Special horticultural practices for early induction of flowering in mango (*Mangifera indica* L.) cv. Ratna

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ABSTRACT

The investigation was conducted at the College of Horticulture, Dapoli, Dist. Ratnagiri, Maharashtra, India during the year 2020-21 to assess the special horticultural practices on induction of flowering in mango (*Mangifera indica* L.) cv. Ratna. The experiment was laid out in Randomized Block Design with three replications and ten treatments *viz*; girdling on the first fortnight of October (T_1), girdling on the first fortnight of November (T_2), girdling on the first fortnight of October and November (T_3), girdling on first fortnight of October and tip pruning (T_4), girdling on the first fortnight of November and tip pruning (T_5), girdling on the first fortnight of October and November and tip pruning (T_6), tip pruning (T_7), removal of new shoots below old shoot (T_8), smudging (T_9) and control (T_{10}). Removal of new shoots (T_7) resulted in early panicle emergence as compared to control. Treatment girdling on the first fortnight of November and tip pruning (T_5) exhibited the highest flowering intensity and hermaphrodite flower per panicle. It also recorded the maximum fruit set and fruit retention per panicle.

***Key words*:** Ratna, tip pruning, girdling, flowering, fruit set.

Mango (*Mangifera indica* L.) is one of the oldest and most popular fruits having the adorable flavour and taste of the tropical world. It belongs to the genus *Mangifera* and the family Anacardiaceae. It is originated from the Indo-Burma region from the genus *Mangifera* almost all the commercial cultivars of mango are included in single species *Mangifera indica* in India. It is the most important tropical fruit in the world. Mango is called the "King of the fruits". It has been variously called *Amra, Atisourabha, Chuta, Sahakara, and Rasala* in ancient Sanskrit literature. Among the various commercial varieties, the variety Ratna was released by DBSKKV, Dapoli (M.S.). The parentage of Ratna is Neelum and Alphonso. The tree is semi-dwarf in growth habit. The fruits are large ovate in shape (400-500 g) with firm and fibreless deep orange colour pulp. It is regular in bearing. It is excellent for processing as well as table purpose. Girdling is the removal of the bark in a circular manner of either branch or trunk of woody plants. Girdling stops the basipetal movement of assimilates through the phloem which results in the accumulation of carbohydrates above the girdle which ultimately helps for induction of early and assured flowering. Urban *et al*. (2009) reported that girdling is one of the ways to improve the earliness and intensity of flowering in mango. The demand for this variety is increasing day by day owing to good keeping quality and spongy tissue-free fruits. The induction of early flowering results in the early maturity of fruits. Such fruits earn greater rates in the market as compared to late-maturing fruits. The weather during the initiation of flowering in the month of October and November play important role in the induction of flowering at the appropriate time. It is often noticed that climatic fluctuations in October-November lead to the production of vegetative flush instead of flowering flush. This new flush requires another 80-100 days to mature as a result, flowering is considerably delayed. Late flowering leads to delayed fruit development and harvesting. The late-harvested fruits fetch low market rates. It is often noticed that many of these new shoots do not produce flowers and hence the flowering is sparse which produces poor yield (Soudagar *et al*., 2018). The young flushes are cut back up to matured wood; the resulting flush can be a floral one. It not only causes a uniform flush of growth throughout the canopy but also removes growth and flower-inhibiting factors in the stem derived from the previous season's flowering and fruiting panicles. Shoot pruning reduce the auxin synthesis at the apex of the branches, directing the transport of assimilates and cytokinin's to the axillary buds of branches under flowering condition, inducing the formation of axillary inflorescences (Srivastava, 2002). Smudging is an ancient method of inducing mango to flower. It is practiced in certain parts of the Philippines to obtain early flowering of 'Carabao' and 'Pico' mango. Ethylene has been identified as the active agent responsible for flowering during smudging (Dutcher, 1972).

MATERIALS AND METHODS

The investigation was conducted on 30 years old mango trees (cv. Ratna) at the College of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (Maharashtra) India, is located between 17°45′ N latitude and 73°12′ E longitude on the West coast of Maharashtra. It has an altitude of 240 m from the MSL. The experiment was laid out in randomized block design with three replications and ten treatments *viz*., girdling on the first fortnight of October (T_1), girdling on the first fortnight of November (T_2), girdling on the first fortnight of October and November (T_3), girdling on the first fortnight of October and tip pruning (T_4), girdling on the first fortnight of November and tip pruning (T_5), girdling on the first fortnight of October and November and tip pruning (T_6), tip pruning (T_7), removal of new shoots below old shoot (T_8), smudging (T_9) and control (T_{10}). Each treatment was given two trees. The girdling was done on tertiary branches of the experimental tree by giving a circular deep cut with help of a sharp knife as per treatments. A total of 50 branches were girdled per experimental plant. On these plants, vegetative shoots emerged in the month of November of the total new shoots. Totally 200 shoots per experimental plant were removed at the point of emergence of mature shoots. The smudging was done in the month of December. During smudging, the colour of newly emerged shoots was light green. Smudging was done early in the morning. On the previous day, materials like rice bran, and dry residues of the plant were collected at the base of the plant canopy. Then next day early in the morning smudging was done for about 2 hours. The entire process of smudging was performed four times at four days intervals. The data on the number of days for induction of flowering, flowering intensity (%), length of panicle (cm), the width of panicle (cm), rachis per panicle, hermaphrodite flowers percentage, fruit set (%) and fruit retention (%), number of days required for flowering to harvesting, yield were recorded. The data were analyzed by using statistical methods suggested by Panse and Sukhatme (1995).

RESULTS AND DISCUSSION

The data on the effect of girdling, tip pruning, and smudging on the induction of flowering presented in Table 1 indicates that they significantly influenced the flowering parameters in mango (cv. Ratna).

Days required for panicle emergence

The early flowering (40.83 days) was observed in tip pruning (T_7) which was significantly superior over the control (131.33 days). Shoot pruning reduces the auxin synthesis at the apex of the branches, directing the transport of assimilates and cytokinins to the axillary buds of branches creating favourable conditions for flowering (Taiz and Zeiger, 2012). Gopu *et al*. (2014) found that minimum days (169 days) were required in the pruned tree as compared to the control (198 days) and showed uniform flowering per panicle in mango cv. Alphonso.

Flowering intensity

The maximum flowering intensity (62.27%) was observed in girdling on the first fortnight of November and tip pruning (T_5) which was significantly superior over all other treatments. The minimum flowering intensity (26.67%) was recorded in the control. Warang *et al*. (2019) reported that maximum flowering intensity was observed by girdling in the first fortnight of September and removal of new shoots (65.67%) as compared to control T_8 (27.33%) in mango cv. Alphonso. Girdling is one of the ways to improve the earliness and intensity of flowering in mango cv. Cogshall (Urban *et al*., 2009). The higher percentage of flowering due to pruning treatments was mainly attributed due to the availability of photosynthetic solar radiation to the leaves which enhanced flowering (Lal and Mishra, 2007).

Panicle length, Panicle width and Number of rachis per panicle

The longest panicle (30.20 cm) was noticed in treatment girdling on the first fortnight of November and tip pruning (T_5) which was at par with T_8 (29.20 cm) and T_6 (28.83 cm). The shortest panicle (24.13 cm) was found in the treatment girdling on the first fortnight of November (T_2). The maximum width of the panicle (24.63 cm) was observed in treatment girdling on the first fortnight of November and removal of new shoots (T_5) and it was at par with T_3 (23.67 cm) and T_4 (22.97 cm). The minimum panicle width (17.27 cm) was found in the control (T_{10}). The highest number of rachis per panicle (31.57) were found in the treatment removal of new shoots below old shoot (T_8) which was at par with T_5 (30.63), T_4 (29.77), T_6 (29.77), T_3 (28.97). The lowest number of rachis per panicle (25.30) was recorded in girdling on the first fortnight of October (T_1) (Table 1). There was an increase in the length and width of panicle as well as the number of rachis per panicle by girdling on the first fortnight of November and tip pruning (removal of new shoots). It may be due to the availability of more sugars and auxins in branches. Nachare (2020) observed the longest length of panicle in girdling on the first fortnight of September (31.37 cm) in mango cv. Ratna. Shoot pruning was significantly effective in increasing the length and width of the panicle. Removal of new shoots leads to the formation of longer panicle lengths due to gross changes in endogenous hormonal levels (Singh *et al*., 2010). Shoot pruning reduces the auxin synthesis at the apex of the branches, directing the

doi: 10.5958/2582-2683.2023.00019.9

Table 1. Effect of girdling tip pruning and smudging on the number of days required for flower induction, flowering intensity and hermaphrodite flower of mango cv. Ratna

Treatments	Days required for panicle emergence	Flowering intensity (%)	Panicle length (cm)	Panicle width (cm)	No. of rachis per panicle
T_1	91.33	38.33	26.30	20.87	25.30
T_2	76.33	43.33	24.13	19.97	28.33
T_3	94.17	41.67	25.10	23.67	28.97
T_4	64.83	59.17	25.20	22.97	29.77
T_5	44.67	62.27	30.20	24.63	30.63
T_6	77.33	60.83	28.83	21.57	29.77
T_7	40.83	52.50	27.67	19.67	26.73
T_8	42.33	42.50	29.20	19.97	31.57
T_9	65.50	32.50	27.47	20.40	27.67
T_{10}	131.33	26.67	25.57	17.27	26.20
C.D. at 5 %	6.63	6.70	1.92	2.73	2.72

Table 2. Effect of girdling tip pruning and smudging on fruit set and fruit retention per panicle of mango cv. Ratna

Treatments	Hermaphrodite flower (%)	Fruit set	Fruit retention (%)	Days required from flowering to harvesting	Yield (No. of fruits/tree)	Yield (kg/ tree)
T_1	12.43	6.30	0.64	150.33	106.67	43.08
T_2	12.17	6.00	0.59	149.67	102.50	41.81
T_3	12.07	5.73	0.57	156.33	101.50	41.48
T_4	14.50	7.30	0.77	147.50	116.33	49.45
T_5	15.01	8.53	0.95	141.07	145.00	61.58
T_6	14.75	8.23	0.87	155.73	134.67	58.10
T_7	13.30	6.70	0.72	134.87	126.33	55.23
T_8	12.10	5.97	0.57	141.67	108.50	48.45
T_9	11.31	5.42	0.55	149.33	100.00	40.52
T_{10}	10.98	5.03	0.50	165.00	88.33	29.47
C.D. at 5 %	1.50	0.33	0.06	7.16	7.89	3.30

transport of assimilates and cytokinin's to the axillary buds of branches, creating favourable conditions for flowering (Taiz and Zeiger, 2012).

Hermaphrodite flower, Fruit set and fruit retention

The data on hermaphrodite flower percentage, fruit set, fruit retention, days required from flowering to harvesting, and yield are presented in Table 2. Treatment T_5 resulted in maximum hermaphrodite flowers (15.01%), and fruit set (8.53%) with 0.95 per cent fruit retention which was significantly superior over control. The highest number of hermaphrodite flowers (%) due to the removal of new shoots was also reported in the earlier studies in mango cv. Alphonso by Gopu *et al*. (2014). The control (untreated) resulted in minimum hermaphrodite flowers (10.98%), minimum fruit set (5.03%), and fruit retention (0.50%). Warang *et al*. (2019) reported that girdling on the first fortnight of September and removal of new shoots produced the highest fruit set per panicle in mango cv. Alphonso. Nachare (2020) also found similar results in mango cv. Ratna.

Days required from flowering to harvest

The minimum days required for flowering to harvest were recorded in treatment T_7 -tip pruning (141.07 days) which was significantly superior among all other treatments. Tip pruning facilitated early flowering and harvesting in mango cv. Ratna. It increases photosynthate translocation to flower buds which results in an earlier fruit set which leads to early harvest than the control (Lal and Mishra, 2007). Soudagar *et al*. (2018) exhibited the minimum number of days required for harvesting in tip pruning by retaining two leaves in mango cv. Alphonso. Similar results were observed by Warang *et al*. (2019) in mango cv. Alphonso and Nachare (2020) in mango cv. Ratna.

Yield

The treatment T_5 recorded a maximum fruit yield of 145 fruits per tree or 61.58 kg per tree. Treatment T_7 required minimum days (134.67 days) from flowering to harvesting which was superior over all other treatments. Girdling can improve carbohydrate availability to fruits and as a

consequence lead to an increased fruit set percentage with decreased bud drop due to branch girdling it also leads to an increase in the number of fruits per shoot and maximum fruit weight which help to increase fruit yield kg per plant and fruit yield kg per hectare (Goren *et al*., 2003). Shinde *et al*. (2015) noticed the highest number of fruits per plant in T_1 (ringing during the first fortnight of May) in cv. Alphonso. Ghadage *et al*. (2017) girdling on 15th July produced a significantly maximum yield (94.20 kg/plant) in mango cv. Alphonso. This may be due to girdling which improved carbohydrate availability to earlier development of fruit and even the removal of new shoots may have stopped the translocation of food to new vegetative growth. The present findings are similar lines with Warnag *et al*. (2019) in mango cv. Alphonso and Nachare (2020) in mango cv. Ratna.

CONCLUSION

The trial concluded that special horticultural practices *viz*., girdling, removal of new shoots (tip pruning), and smudging in mango cv. Ratna was beneficial for the early induction of flowering and early harvesting. Among all treatments, T_7 (removal of new shoots) was best for early induction of flowering and early harvesting. Treatment T_5 (girdling on the first fortnight of November and tip pruning) was best for the highest hermaphrodite flowers, maximum fruit set, and retention and also contributed to the highest yield with greater appreciation with respect to rate in the market.

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Online Manuscript No. 98424 recived on 2.6.2022
Manuscript accepted for publication on 18.11.2022

Journal of Eco-friendly Agriculture **18**(1): 119-124. 2023
doi: 10.5958/2582-2683.2023.00020.5

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Effect of plant-based products on weed management in sunflower

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ABSTRACT

Field experiment conducted at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during *kharif* 2019 to screen out the best among several plant based products *viz*., live mulching with sunhemp and navathaniyam (multi-varietal crops), dried sunflower stalk @ 4 t ha^{-1}, farmer's market vegetable waste @ 4 t ha^{-1}, *Terminalia chebula* powder @ 400 kg ha^{-1}, tamarind leaf mulch @ 4 t ha^{-1}, mango leaves @ 4 t ha^{-1}, eucalyptus leaves @ 7 t ha^{-1}, teak leaves @ 4 t ha^{-1}, rice husk @ 2 t ha^{-1}, mustard seed powder @ 160 kg ha^{-1}, neem leaves @ 2.5 t ha^{-1}, *Butea frondosa* flowers and leaves @ 4 t ha^{-1}, banana leaf mulch @ 7 t ha^{-1} and water hyacinth @ 4 t ha^{-1}, evaluated against sunflower weeds, showed that the eucalyptus and mango leaves mulchin at 7 t ha^{-1} and @ 4 t ha^{-1}, respectively were found significant in delaying the emergence of weeds upto 17 DAS and also were best in reducing the weed density, weed dry weight better plant growth and improved soil quality. The eucalyptus leaves were also found best in producing significantly higher dry matter production (630 kg ha^{-1}) followed by mango leaves (628 kg ha^{-1}) due to the allelopathic effect on weeds suppressing its growth and proliferation.

Keywords: Sunflower, organic mulching, allelopathy, eucalyptus leaf mulch, mango leaf mulch.

Sunflower (*Helianthus annuus* L.) is an essential oilseed crop in the arid and semi-arid regions. It is the main source of edible oil all over the globe as it is rich in poly unsaturated fatty acids (PUFA). Sunflower by virtue of its short duration, wider adaptability to different soil types, thermo and photo-insensitivity and availability of promising hybrids and varieties, high yield potential, profitability has stabilized its area and production in India (Sarkar *et al*., 2005). Sunflower oil is preferred among the consumers world-wide due to its health appeal. It is cultivated in 0.51 million hectare with a production of 5.85 million tonnes. There is a wider scope for improving the sunflower yields by correcting many constrains that affect its productivity. The weeds among them, which compete for space, soil moisture, light and nutrients, are the major threat, resulting in seed yield loss from 45 to 65 per cent (Wanjari *et al*., 2001, Legha *et al*., 1962).

Ignorance of weed growth causes enormous loss of nutrients. Reduction in yield depends on various aspects like weed density, time and duration of weed competition (Kruidhof *et al*., 2008). The critical period of weed competition was found to be of 20 to 49 days after sowing (Wanjari *et al*., 2000). Though, sunflower crop is poor competitor against weeds because of its slow growth at early stage, it does not cover the ground early enough to prevent weed establishment. Therefore, early season weed control against annual weeds is essential for good yields. On the contrary, large scale utilization of chemicals for weed control has resulted in deterioration of soil fertility, residual toxicity, development of resistance by weeds, loss in biodiversity, increasing cost of herbicides and decrease in sustainability paved way for environmental pollution.

Under these circumstances, the concept of allelopathy is one of the safe alternatives to overcome these problems and to achieve sustainability in agriculture and maintenance of healthy environment for future generations. Trees produce ample amount of litter, which are potential source of allelochemicals. The sunflower species are allelopathic in nature. The cultivated sunflower has great allelopathic potential and inhibits weed-seedling growth of velvet leaf, morning glory, wild mustard and other weeds (Macías *et al*., 1998). There is vast possibility of increasing the existing levels of sunflower production and productivity through biomulching in varied ecosystems. Mulches are the safe option that acts directly on stages in the life cycle of weeds by preventing seed germination and also reducing the emergence of new weeds entering the soil seedbank underneath. Spreading a layer of the allelopathic biomulches to 2 to 3-inch mulch layer on the soil would not only reduce moisture loss through evaporation but also hinders weeds (Bond and Grundy, 2001). These biomulches are also quite productive in improving soil quality by retention of soil moisture, prevention of leaching, improving

soil structure further significant effects on soil respiration, microbial biomass, organic matter content and nutrient content. It can also help in reducing water erosion by acting as soil cover for a shorter period of time. Based on this, the present study was formulated to evaluate various bio-mulches and its effect on weed suppression, delayed weed emergence and growth potential of sunflower.

MATERIALS AND METHODS

The field experiment was conducted in the eastern block farm (11°N and 77°E) of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during *kharif* (July to September) of 2019. The soil was sandy clay loam in texture and taxonomically classified as *Typic ustropept* with clay (37.0%), silt (20.1%), fine sand (18.7%) and coarse sand (24.2%). The soil of the experimental field was neutral in pH with 0.50 organic carbon, available N (215 kg ha^{-1}), P_2O_5 (16.7 kg ha^{-1}) and K_2O (525.3 kg ha^{-1}) content, respectively. The annual rainfall of 328.4 mm distributed over 28 rainy days, maximum and minimum temperatures ranged between 29.6- 33.8°C and 20.5 °C and 23.9°C, mean relative humidity ranged from 84.3 to 90.0 per cent (1422 hours) to 49.0 to 70.0 per cent (0722 hours), mean bright sun shine hours of 3.7 to 10.5 hrs day^{-1} open pan evaporation values ranged from 4.2 to 6.6 mm day^{-1} whereas, average sunshine hours varied from 2.9 to 5.8 hours day^{-1} respectively persisted during the experimental period.

Composite soil samples were collected at a depth of 15 cm randomly in the experiment field before sowing for identifying soil texture using Robinson's International pipette method (Piper, 1966), pH using 1:2 soil water suspension (Jackson, 1973), soil organic carbon (%) using Wet chromic acid digestion method (Walkley and Black, 1934), organic matter, available nutrients like Available N using Alkaline permanganate method (Subbaih and Asija, 1956), P_2O_5 using Olsen's method and Available K_2O content using Neutral normal ammonium acetate method (Stanford and English, 1949).

Sunflower (*Helianthus annuus* L.) crop hybrid TNAU SFH CO 3 with a crop duration of 90 days was chosen for the experiment. The experimental field was laid out in Completely Randomised Design encompassing sixteen treatments with three replicates during *Kharif* 2018. The experimental field was ploughed with disc plough followed by cultivator and pulverized later with rotavator to bring the soil to a fine tilth. FYM @ 12.5 t ha^{-1} was evenly spread in the field and micro plots of size 1 m x 1 m. Beds and drainage channels were formed followed by rectification of bunds. Sunflower seeds were sown and all the biomulches were applied as per the treatment schedule. First irrigation was given immediately after sowing. One life saving irrigation was given at 3 DAS. Subsequent irrigations were given at weekly interval.

Weed emergence was observed on daily basis upto 21 DAS and their germination was recorded. The weed present in the experimental plots were morphologically identified and categorized as grasses, sedges and broad leaved. Weed density was recorded using quadrant (0.5 x 0.5m) randomly at four places in each plot upto 21 DAS. This was averaged and expressed as No. per m^2. Relative density was calculated based on the number of individual weed species and total number of weeds. Once the weed density was recorded, weed samples were collected on 21 DAS, shade dried and oven dried for 72 hrs at 70 ^oC till constant weight was obtained and expressed as g m^{-2}. The weed control efficiency was computed based on the weed dry weight in the field at 21 DAS using the formula suggested by Mani *et al.* (1973). From each plot, three plants were selected randomly and tagged for recording biometric observations upto 21 DAS as outlined by (Johnson *et al*., 1981). The height of tagged plants was recorded from base to the tip of fully opened terminal leaf on 21 DAS. Later, average was calculated for the three plants and expressed in cm. The actual number of leaves produced by the individual plant was counted among the three tagged plants. The mean value of the number of leaves per plant was calculated and expressed in No. per plant on 21 DAS. The leaf area of the index for each of three tagged plants was computed using leaf area meter and was expressed in cm^2 $plant^{-1}$. The sunflower plants were harvested at 3 WAS, shade dried and weighed for dry matter production and expressed in kg ha^{-1}.

The data on weed parameters and crops were transformed by via (“X + 0.5) transformation suggested by (Gomez and Gomez, 1984) to point out the variation in treatments and to find out the treatment effect. The transformed data pertaining to crop growth, weed and yield parameters were statistically analysed using Fischer's method of ANOVA. The critical differences were calibrated at probability level of five per cent. The experimental data on growth attributes were analysed statistically using AGRES (Data entry module for Agress statistical software version 3.01, 1994 Pascal Intl. Software Solutions) by Analysis of Variance (ANOVA) suggested by (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Response of weed parameters in sunflower to biomulching

The weed emergence was considerably affected by biomulches and is presented in Table 1. The weeds started emerging after the 17 DAS. Application of eucalyptus leaves

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00020.5

mulch at 7 t ha^{-1} (T_8) and mango leaves at 4 t ha^{-1} was quite effective in delaying the weeds emergence upto 17 DAS. Tamarind leaf mulch at 4 t ha^{-1} and *Terminalia chebula* powder at 400 kg ha^{-1} delayed the emergence of weeds upto 14 DAS. Other biomulches were moderate in controlling weeds under check upto 12 DAS. The weeds started emerging from 4 DAS in unmulched control plot. The pattern of germination of weeds at different days after biomulching was noted down up to 21 DAS. Mulching with eucalyptus leaves at 7 t ha^{-1} showed better results by delaying the emergence of weeds present underground in weed seed bank of soil. Eucalyptus leaves and mango leaves at 4 t ha^{-1} treated plot recorded weed free condition upto 17 DAS. Concomittant results were obtained by (El-Rokiek *et al.,* 2010) soil applied with mango leaf powder and aqueous extract completely inhibited the sprouting of purple nut sedge tubers and significant reduction in the growth of mother shoots was found. These results were also in corroboration with (Puig *et al.*, 2013).

Weed Flora

The weed flora of experimental field consisted of *Chloris barbata*, *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Dinebra retroflexa* and *Eleusine indica* under grasses. *Amaranthus viridis*, *Boerhaavia diffusa*, *Digeria arvensis*, *Parthenium hysterophorus* and *Trianthema portulacastrum* under broad-leaved weeds. Amid ifferent treatments imposed the grasses, sedge and broad leaved weeds density was significantly reduced due to adopted biomulches. *Cynodon dactylon* was found primarily in this field which might be due to continuous proliferation of vegetative propagules, environmental conditions favourable for multiplication of *Cynodon dactylon* without any interruption for many seasons. This was in conformity with the findings of Renukaswamy *et al.* (2012).

Weed density

At 21 DAS of sunflower, eucalyptus leaves biomulch at 7 t ha^{-1} (T_8) lowered the number of weeds (1.9 /m^2) followed by mango leaves at 4 t ha^{-1} (3.8 /m^2). It was comparable with *Terminalia chebula* powder at 400 kg ha^{-1} (3.3 /m^2) followed by tamarind leaf mulch at 4 t ha^{-1} (3.8 /m^2) recorded lower density. Moderately lower density of weeds was found in live mulching with sunhemp at 40 kg ha^{-1} (4.7 /m^2) and live mulching with multi-crops (Navathaniyam) at 50 kg ha^{-1} (5.2/m^2). Control (unmulched) plot had recorded higher density (24.8 /m^2). Due to imposition of weed control treatments had hysterically influenced on density of different weed species over un-mulched (un-weeded check). Distinct decrease in total weed density was noted with application of eucalyptus leaves at 7 t ha^{-1} which was on par with mango leaves at 4 t ha^{-1}. This might be due to suppression of weed germination by the allelechemicals present in eucalyptus and mango leaves. Similar results were in congruency with the findings of El-Rokiek *et al.* (2010). He reported that mango leaves contained phenolic components that had herbicidal potential that could control sedges especially purple nut sedge.

Weed dry weight

Application of eucalyptus leaves at 7 t ha^{-1} (T_8) (2.4 g /m^2) lowered the number of weeds followed by mango leaves at 4 t ha^{-1} (2.8 g /m^2) was comparable with *Terminalia chebula* powder at 400 kg ha^{-1} (6.0 g /m^2) followed by tamarind leaf mulch at 4 t ha^{-1} (6.6 g /m^2) recorded lower weed dry weight at 3WAS of sunflower. Moderately lower weed dry weight

Table 1. Effect of biomulches on weed emergence pattern of all weeds species in sunflower

Treatments	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS	12 DAS	14 DAS	16 DAS	18 DAS	20 DAS
T1	0	0	0	0	0	0	0	2	2	1
T2	0	0	0	0	0	0	0	3	2	0
T3	0	0	0	0	0	0	0	0	0	0
T4	0	0	0	0	0	0	0	0	0	0
T5	0	0	0	0	0	0	0	3	0	0
T6	0	0	0	0	0	0	0	2	2	0
T7	0	0	0	0	0	0	0	0	2	0
T8	0	0	0	0	0	0	0	0	1	0
T9	0	0	0	0	0	0	2	4	2	0
T10	0	0	0	0	0	0	3	4	2	0
T11	0	0	0	0	0	0	1	3	1	1
T12	0	0	0	0	0	0	1	1	1	1
T13	0	0	0	0	1	3	5	1	0	0
T14	0	0	0	0	1	6	2	0	0	0
T15	0	0	0	0	4	4	2	1	0	0
T16	0	5	6	7	0	1	4	1	0	0

Data is not statistically analysed

doi: 10.5958/2582-2683.2023.00020.5

was found in live mulching with sunhemp at 40 kg ha^{-1} (8.3 g /m^2) and live mulching with multi-crops (Navathaniyam) at 50 kg ha^{-1} (9.0 g /m^2). Control (unmulched) plot had recorded higher density (34.4 g /m^2). These findings were in line with Puig *et al.* (2013). Eucalyptus leaves suppressed the seedling growth and establishment of vegetative propagules of weeds like *Cyandon dactylon* L. were in the view of with Babu and Kandasamy (1997). Similar findings were in confirmation with El-Rokiek *et al.* (2010). This might be due to presence of alkaloids like coumarin, o-coumaric acid, p-coumaric acid, benzoic acid, p-hydroxybenzoic acid, ferulic acid and cinnamic acid.

Weed control efficiency

Weed control efficiency of 100 per cent was registered highest with application of dried sunflower stalk at 4 t ha^{-1} whereas eucalyptus leaves at 7 t ha^{-1}, mango leaves at 4 t ha^{-1} registered higher weed control efficiency more than 93.0 % and 91.9% during phase I. Live mulching with sunhemp at 40 ha^{-1} and multi-varietal crops (Navathaniyam) at 50 kg ha^{-1} had recorded noticeably higher weed control efficiency of 70 per cent. Amid of different treatments, lower weed control efficiency of less than 50 per cent was recorded in T_9, T_{10}, T_{11} and T_{12} at all stages of crop. This could be due to suppression of weed emergence and growth by release of many volatile germination inhibitors like p-coumaric acid, caffeic acid upon decomposition of mulches were detrimental to the weeds.

Growth attributes of irrigated sunflower

Plant height, number of leaves per plant and leaf area index

At third week of sunflower, unwedded control (unmulched) recorded significantly lower plant height (28.7 cm) whereas eucalyptus leaves treated plot at 7 t ha^{-1} (T_8) (45.5 cm) was comparable with Mango leaves at 4 t ha^{-1} (T_7) (45.5 cm) followed by *Terminalia chebula* powder at 400 kg ha^{-1} (T_5) (41.3 cm) on par with tamarind leaf mulch at 4 t ha^{-1} (41 cm). Application of eucalyptus leaves at 7 t ha^{-1} (T_8) and mango leaves at 4 t ha^{-1} (T_7) produced eight leaves per plant and did not show much significance. Unwedded control plot recorded significantly lower number of leaves at 14 DAS. The leaf area index was significantly higher in eucalyptus leaves at 7 t ha^{-1} (T_8) (0.97), mango leaves at 4 t ha^{-1} (0.95) was followed by tamarind leaf mulch at 4 t ha^{-1}. LAI was considerably lower in unweeded control (0.42 and 1.02). Application of bio-mulches produced distinct variations on the plant height during the entire crop period. Taller plants were produced in eucalyptus leaves at 7 t ha^{-1} treated plot

Table 2. Effect of biomulches on weed density and dry weight of irrigated sunflower at 21DAS.

Treatments	Total grass dry weight (g m^{-2})	Total Broad leaved dry weight (g m^{-2})	Total weed dry weight (g m^{-2})	Total grass dry weight (g m^{-2})	Total Broad leaved dry weight (g m^{-2})	Total weed dry weight (g m^{-2})	Weed control efficiency (WCE) (%)
T_1: Live mulching with sunhemp @ 40 kg ha^{-1}	1.87 (3.0)	1.48 (1.7)	2.28 (4.7)	2.41 (5.3)	1.87 (3.0)	3.0 (8.3)	75.9
T_2: Live mulching with Multi varietal Crops (Navathaniyam) @ 50 kg ha^{-1}	1.95 (3.30)	1.55 (1.9)	2.39 (5.2)	2.47(5.6)	1.97 (3.4)	3.1 (9.0)	73.8
T_3: Dried sunflower stalk @ 4 t ha^{-1}	0.71 (0.0)	0.71 (0.0)	0.70 (0.0)	0.70(0.0)	0.70 (0.0)	0.70 (0.0)	100.0
T_4: Farmers market vegetable waste @ 4 t ha^{-1}	1.58 (2.0)	0.71 (0.0)	1.58 (2.0)	1.82(2.8)	0.70(0.0)	1.8 (2.8)	91.9
T_5: *Terminalia chebula* powder @ 400 kg ha^{-1}	1.61 (2.1)	1.30 (1.2)	1.95 (3.3)	2.12(4.0)	1.58 (2.0)	2.5 (6.0)	82.6
T_6: Tamarind leaf mulch @ 4 t ha^{-1}	1.70 (2.4)	1.38 (1.4)	2.07 (3.8)	2.17(4.2)	1.70 (2.4)	2.7 (6.6)	80.8
T_7: Mango leaves @ 4 t ha^{-1}	1.55 (1.9)	0.71 (0.0)	1.55 (1.9)	1.82(2.8)	0.70 (0.0)	1.8 (2.8)	91.9
T_8: Eucalyptus leaves @ 7 t ha^{-1}	1.30 (1.2)	0.71 (0.0)	1.30 (1.2)	1.70(2.4)	0.70 (0.0)	1.7 (2.4)	93.0
T_9: Teak leaves @ 4 t ha^{-1}	2.41 (5.3)	1.84 (2.9)	2.95 (8.2)	2.74 (7.0)	2.26 (4.6)	3.5 (11.6)	66.3
T_{10}: Rice husk @ 2 t ha^{-1}	2.53 (5.9)	1.87 (3.0)	3.07 (8.9)	2.77 (7.2)	2.30 (4.8)	3.5 (12.0)	65.1
T_{11}: Mustard seed powder @ 160 kg ha^{-1}	2.14 (4.1)	1.67 (2.3)	2.63 (6.4)	2.65 (6.5)	2.19 (4.3)	3.4 (10.8)	68.6
T_{12}: Neem leaves @ 2.5 t ha^{-1}	2.17 (4.2)	1.73 (2.5)	2.68 (6.7)	2.70(6.8)	2.00 (3.5)	3.3 (10.3)	70.1
T_{13}: *Butea frondosa* flowers and leaves @ 4 t ha^{-1}	2.55 (6.0)	1.95 (3.3)	3.13 (9.3)	2.93(8.1)	2.34 (5.0)	3.7 (13.1)	61.9
T_{14}: Banana leaf mulch @ 7 t ha^{-1}	2.61 (6.3)	2.00 (3.5)	3.21 (9.8)	3.00(8.5)	2.41 (5.3)	3.8 (13.8)	59.9
T_{15}: Water hyacinth @ 4 t ha^{-1}	2.85 (7.6)	2.07 (3.8)	3.45 (11.4)	3.45(11.4)	2.49 (5.7)	4.2 (17.1)	50.3
T_{16}: Weedy check @ Unmulched plot	4.00 (15.9)	3.10 (8.9)	5.00 (24.8)	4.00(15.9)	4.40 (18.5)	5.9 (34.4)	0.0
SEm±	0.09	0.07	0.11	0.10	0.08	0.13	-
CD (P=0.05)	0.19	0.14	0.23	0.22	0.18	0.27	-

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00020.5

Table 3. Effect in biomulches on growth attributes of irrigated sunflower at 21DAS

Treatments	Plant height (cm)	Number of leaves	Leaf area index	Dry matter production (kg ha^{-1})
T_1: Live mulching with sunhemp @ 40 kg ha^{-1}	40.8	7	1.70	570
T_2: Live mulching with Multi varietal Crops (Navathaniyam) @ 50 kg ha^{-1}	40.6	7	1.69	567
T_3: Dried sunflower stalk @ 4 t ha^{-1}	0.0	0	0.00	0.00
T_4: Farmers market vegetable waste @ 4 t ha^{-1}	0.0	0	0.00	0.00
T_5: *Terminalia chebula* powder @ 400 kg ha^{-1}	41.3	7	1.74	574
T_6: Tamarind leaf mulch @ 4 t ha^{-1}	41.0	7	1.72	572
T_7: Mango leaves @ 4 t ha^{-1}	45.5	8	1.91	628
T_8: Eucalyptus leaves @ 7 t ha^{-1}	45.5	8	1.92	630
T_9: Teak leaves @ 4 t ha^{-1}.	37.3	7	1.48	492
T_{10}: Rice husk @ 2 t ha^{-1}	37.0	7	1.46	490
T_{11}: Mustard seed powder @ 160 kg ha^{-1}	37.4	6	1.50	511
T_{12}: Neem leaves @ 2.5 t ha^{-1}	37.4	6	1.49	505
T_{13}: *Butea frondosa* flowers and leaves @ 4 t ha^{-1}	33.0	5	1.26	433
T_{14}: Banana leaf mulch @ 7 t ha^{-1}	32.6	5	1.25	425
T_{15}: Water hyacinth @ 4 t ha^{-1}	32.5	5	1.22	420
T_{16}: Weedy check @ Unmulched plot	28.7	4	1.02	360
SE m±	1.40	0.24	0.05	19.73
CD (P=0.05)	2.86	0.49	0.11	40.13

comparable with mango leaves at 4 t ha^{-1} followed by tamarind leaves at 4 t ha^{-1}. This might be due to better accumulation of all the resources, synthesis of all photosynthates required for plant growth both horizontally and vertically. The competition free conducive environment led to higher nutrient uptake resulting in higher leaf area index, plant height and dry matter production in sunflower (Wanjari *et al*., 2001).

Dry matter production (DMP)

Dry matter production of crop as affected by different biomulches during study was recorded were represented in the Table 3. Significantly higher dry matter of crop were recorded in eucalyptus leaves at 7 t ha^{-1} (630 kg ha^{-1}) and mango leaves at 4 t ha^{-1} (628 kg ha^{-1}) during experiment. Moderate dry matter of crop were recorded in Tamarind leaf mulch at 4 t ha^{-1} (572 kg ha^{-1}) and *Terminalia chebula* powder at 400 kg ha^{-1} (574 kg ha^{-1}). The results showed that the dry matter of crops (360 kg ha^{-1}) were significantly less in control during the cropping period. Weeds compete with crops for resources like water, nutrients, space and light. Therefore, cause significant reduction in yields upto 55% (Wanjari *et al*., 2001). Nevertheless, there is possibility of increasing sunflower yields by creating weed free environment (Wanjari *et al*., 2001). These factors could be addressed taking into consideration that sunflower has poor growth at early stages. Consequently, timely removal of weeds by virtue of keeping them under check is indispensable. Keeping this in mind bio-mulches were adopted for prolonged weed management of sunflower in a sustainable manner other than chemical weed control.

CONCLUSION

The study concludes that biomulching with eucalyptus leaves @ 7 t ha^{-1} was found the best in controlling the broad spectrum of weeds with higher weed control efficiency, lower weed density, weed dry weight and keeping weed free condition for longer period of time upholding the theme "Best out of waste practice".

ACKNOWLEDGEMENTS

The authors express their gratitude to the Department of Agronomy and Department of Sustainable Organic Agriculture, Tamil Nadu Agriculture University, Coimbatore, India for facilitating chemicals, implements and instruments for conducting the laboratory and field studies.

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Online Manuscript No. 97655 recived on 14.4.2022
Manuscript accepted for publication on 10.11.2022

Journal of Eco-friendly Agriculture **18**(1): 125-129. 2023
doi: 10.5958/2582-2683.2023.00021.7

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Effect of nuclear polyhedrosis virus on botanicals treated larvae of *Bombyx mori* L.

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ABSTRACT

Laboratory testing of virulence of two BmNPV stains KRI 1 and DMI 1 tested against multivoltine silkworm breeds showed that the KRI-1 stain showed high mortality of 75.03 per cent in Pure Mysore followed by Tamil Nadu White (73.66%) while C. Nichi recorded minimum of 61.0 per cent mortality. Another study conducted on the efficacy of botanicals against multivoltine breeds, *viz*., Nistari, C. Nichi and Tamil Nadu White infected with BmNPV revealed that *Plectranthus amboinicus* recorded significantly lesser mortality of 34.60 per cent, 30.10 per cent and 33.00 per cent, respectively and was on par with *Psoralea corylifolia* (35.65%, 29.33%, 34.00%) against the mortality of 60.00 per cent (Nistari), 59.00 per cent (C. Nichi) and 52.00 per cent in Tamil Nadu White, respectively. Results on the response of the above breeds to a particular treatment revealed that mortality was significantly lowest in C. Nichi for both the treatments, *P. corylifolia* (29.00%) and *P. amboinicus* (31.10%), followed by Nistari (34.60%, 35.65%).

Keywords: Sericulture, Silkworm larva, multivoltine breeds, Nistari, C.Nichi, Tamil Nadu White, NPV, Botanicals, *P. amboinicus, P. corylifolia*

The mulberry silkworm, *Bombyx mori* L., is of great economic importance in many silk-producing countries of the world (Krishnaswami *et al*., 1992). Four silkworm diseases, namely Grasserie (viral), Flacherie (bacterial), Muscardine (fungal), and Pebrine (protozoan), are common in India. Among these the nuclear polyhedrosis caused by nuclear polyhedrosis virus (BmNPV) is the most serious in tropical countries due to its prevalence throughout the year. It is an ubiquitous disease with varying severity different regions (Sengupta *et al*., 1990). A survey in Tamil Nadu revealed that the loss due to nuclear polyhedrosis virus ranged from 0.5 to 22.5 per cent in Coimbatore, Salem, Erode, Dharmapuri, and Krishnagiri districts (Sivaprakasam, 1994a). Now, eco-friendly methods decline the viral diseases of silkworm for plant molecules are appropriate for the current scenario because of their cost-effectiveness and eco-friendly nature. Besides the antimicrobial effects, the growth-promoting factors of botanicals were also demonstrated. The exploitation of such antiviral compounds from plants will be of greater use in the sericulture industry as they are ecologically safe for use under a controlled environment like a rearing room and its easier availability. Plant products are cheaper besides facilitating easier application (Rajasekhar Gouda, 1991; Manimegalai and Chandramohan, 2006a). The present study was aimed to investigate the effect of BMNPV in silkworm larva and the impact of botanicals in BMNPV in multivoltine silkworm larvae.

MATERIALS AND METHODS

Investigations were carried out at the Department of Agricultural Entomology, Adhiyamaan College of Agricultural and Research, Athimugam, Krishnagiri, Tamil Nadu during 2019-2020. Silkworm rearing was carried out in an adequately disinfected rearing house at 25°C and 80 per cent relative humidity. The disease-free laying (Dfls) were procured from various central sericulture institutions and the state department of sericulture, Tamil Nadu, and Karnataka. Multivoltine silkworm breeds *viz*, Pure Mysore, C. Nichi, Nistari and Tamil Nadu white were used. Milk disease infected fifth instars silkworm were collected, washed, and macerated with pestle and mortar. Virus suspension so obtained was filtered using a muslin cloth. The filtrate was centrifuged at 500 rpm for 30 seconds and the supernatant was again centrifuged at 3000 rpm for three minutes. The supernatant was then discarded, and the pellets obtained were suspended in distilled water and stored in the refrigerator at 4°C for bioassay studies (Sugumori *et al.,* 1990). Nuclear polyhedral occlusion bodies in the suspension were counted with the help of an improved neubauer haemocytometer using a phase-contrast microscope.

For preparation of extracts, seeds of *Psoralea corylifolia* (a medicinal plant known as *Bakuchi* or *Babchi* in hindi) and leaves of *Plectranthus amboinicus,* an aromatic succulent herb

femous for its oregno like flovor were used. Seeds of *P. corylifolia* were dried under shade and powdered. Leaves of *P.amboinicus* were thoroughly washed under running tap water, rinsed twice with distilled water, dried under the shade and then powdered. For six hours, the powdered seed and leaf samples were weighed and extracted with hexane in a soxhlet apparatus (Khatune, 2000). These materials were separately kept at room temperature for evaporation of solvents. The residue was weighed and dissolved in an equal volume of acetone (W/V) to get a working stock solution. The required concentration of 1000 ppm was prepared using distilled water.

Two strains of BmNPV *viz*., KNI 1 (Krishnagiri) and DMI 1 (Dharmapuri), were tested for their virulence against multivoltine breeds of *B. mori* to identify the most virulent one for bioassays. Fresh mulberry leaves were dipped in a 10^6 POBs ml^{-1} viral suspension and shade dried. Fifty third instar larvae each of silkworm breeds viz., Pure Mysore, C. Nichi, Nistari, and Tamil Nadu, were used for the bioassay studies in four replications. The worms after the second moult were fed with mulberry leaves treated with virus @10^6POBs ml^{-1}.

Observations on larval mortality, larval weight, cocoon weight and shell weight, shell ratio and survival percentages were recorded and computed.

RESULT AND DISCUSSION

Pathogenicity of BmNPV strains against different breeds of B. mori

The virulence of two strains of BmNPV, *viz*., KNI 1 and DMI 1, were studied against multivoltine breeds of *B. mori* for further use of any one of them for further studies on testing the efficacy of botanicals.

The result revealed that BmNPV infection larval duration was prolonged in C.Nichi (155 h.), and was on par with Pure Mysore (146 h.) and Nistari (146 h.) followed by TN White (142 h.). KNI 1 strain of BmNPV produced significantly higher mortality in Pure Mysore (75.03 %) and was on par with other breeds (Table 1). C.Nichi breed of *B. mori* recorded significantly the highest larval weight of 1.03 g, followed by Nistari (0.97 g), TN White (0.94 g), and Pure Mysore (0.89 g) recorded significantly lower larval weight, and both were on par with each other. Cocoon weight was significantly higher in Pure Mysore (0.56 g) and was on par with TN White (0.52 g) and C. Nichi (0.50 g), followed by Nistari (0.40g), recorded significantly lower cocoon weight and was on par with each other. C.Nichi (0.09g) recorded significantly highest shell weight followed by Pure Mysore (0.06 g), TN White (0.06 g), and Nistari (0.05 g), and all were on par with each other. Nistari recorded the highest shell ratio of 16.35 per cent and was on par with C.Nichi (15.00 %), followed by TN White (13.29 %) and Pure Mysore (12.09 %), and all were on par with each other (Table 1).

The results on DMI strain of BmNPV; larval duration (h.) and mortality (%) showed that BmNPV infection, larval duration was longer in C.Nichi (160 h.). The breeds, *viz*. Nistari (150 h.), TN White (145 h.), Pure Mysore (142 h.), and all were found to be on par with each other. DMI strain of BmNPV produced significantly higher mortality in Nistari (70.23 %) (Table 2). The result showed that, the economics parameters (Larval weight, cocoon weight, shell weight and shell ration) mentioned as on below (Table 2). C.Nichi breed of *B. mori* recorded the highest larval weight of 1.03 g significantly. The breeds Nistari (0.97 g) and Pure Mysore (0.86 g) were significantly next best to C.Nichi, and all were found to be on par with each other, followed by TN White (0.78 g). The cocoon weight was significantly higher in C.Nichi (0.60g) followed by Pure Mysore (0.55g), TN White (0.50 g), Nistari (0.49 g), and all were on par with each other. C.Nichi recorded significantly highest shell weight of 0.09 g, followed by Nistari (0.06 g), TN White (0.05 g), Pure Mysore (0.05 g), and all the above breeds were on par with each other. Nistari recorded the highest shell ratio of 13.55 per cent and was on par with C.Nichi (12.00 %), which in turn was on par with Pure Mysore (11.53 %), followed by TN White (9.33 %). The discussion for the result as the tolerant nature of multivoltine breeds to stress conditions, they are

Table 1. Effect of BmNPV (KNI 1 strain) on larval duration (h.), larval mortality (%) and economic parameters of multivoltine breeds of *B. mori*.

Sl. No.	Breeds	Larval duration (h.)	Mortality (%)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
1.	Nistari	146^{b}	66.00 ^{b}	0.97 ^{b}	0.40 ^{d}	0.05 ^{bc}	16.35 ^{a}
2.	C.Nichi	155 ^{a}	61.00 ^{c}	1.03 ^{a}	0.50 ^{ab}	0.09 ^{a}	15.5 ^{a}
3.	Pure Mysore	146^{a}	75.03 ^{a}	0.89 ^{c}	0.56 ^{a}	0.06^{b}	12.09 ^{b}
4.	TN White	140 ^{a}	73.66 ^{a}	0.94 ^{b}	0.52 ^{ab}	0.06 ^{b}	13.29 ^{b}
	SEd	2.2251	1.4241	0.0330	0.0231	0.0086	0.9841
	CD (P=0.05)	4.5133	2.9331	0.0590	0.0583	0.0135	2.1226

In a column, means followed by same letter (s) are not significantly different (P=0.05).

doi: 10.5958/2582-2683.2023.00021.7

more suited to Indian Sericulture than the bivoltine and hence India is mostly multivoltine oriented (Maribashetty *et al.,* 1998). The present result is strengthened by Muni Krishnappa *et al.* (2002) report that traditional races had higher crop stability. Koundinya *et al.* (2009) opined that multivoltine breeds are primarily indigenous in origin and show resistance to adverse climatic conditions and tolerance to disease. Sivaprakasm and Rabindra (1997) reported that the breeds C.Nichi and TN White were less susceptible. C.Nichi was found to be tolerant to BmNPV. The present result corroborates with Nirmal Kumar *et al.* (1998), who reported a negative correlation between cocoon shell ratio and pupation rate in pure races.

Efficacy of botanicals on mortality and economic parameters of *B. mori* exposed to BmNPV

Larval mortality (%) *of Multivoltine breeds (Nistari, C. Nichi* and *TN white)* Among the treatments, *P. amboinicus* recorded the lowest mortality of 34.60 per cent and was on par with *P. corylifolia* (35.65%). Treated control recorded the highest mortality of 60 per cent (Table 5). Results on the response of three breeds to a particular treatment revealed that mortality was significantly lowest in C.Nichi for both the treatments, *P. corylifolia* (29.00 %), and *P. amboinicus* (30.10 %), followed by Nistari (34.60 %, 35.65 %) for the above treatments (Table 3). Survival of larvae (%) in *P. corylifolia* was the highest (63.00%) followed by *P. amboinicus* (65.00%). The larval survival of *C. Nichi* was significantly highest in *P. amboinicus.* (70.00 %) and *P. corylifolia* (69.10%) while the larval survival of TN White (%) was maximum in *P.amboinicus* (68.20%) and *P. corylifolia* (66.85%) followed by treated control (55.00%). Significantly similar response was observed in all the three breeds tested, *viz.,* Nistari (63.00 %), C. Nichi (69.70 %) and TN White (66.88 %) for the treatment, *P. corylifolia*. For the treatment, *P. amboinicus*, C. Nichi (70.50 %), and TN White (68.20 %) responded equally well followed by Nistari (63.50 %) (Table 3). Data on duration of larvae (h) revealed that the breed C. Nichi recorded significantly shortest larval duration for the treatments, *P. corylifolia* (141h.) and *P. amboinicus* (142 h.) followed by TN White (148h., 149 h.) and Nistari (162 h., 160 h.) for the above treatments, respectively. C. Nichi recorded the shorter larval duration of 141 h. followed by TN White (153 h.) and Nistari (158 h.) for the treated control. No significant difference in larval duration was observed between breeds in case of untreated control (Table 4).

The data on larval weight among differents breeds for a particular treatment revealed that C. Nichi recorded significantly highest larval weight for both *P. corylifolia* (1.10 g) and *P. amboinicus* (1.07 g), followed by Nistari (1.00g and 0.99g) and TN White (1.00 and 0.97 g) which in turn were on

Table 2. Effect of BmNPV (DMI 1 strain) on larval duration (h.) and larval mortality (%), economic parameters of multivoltine breeds of *B. mori*.

Sl. No.	Breeds	Larval duration (h)	Mortality (%)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
1.	Nistari	150b ^{c}	70.23 ^{a}	0.97 ^{b}	0.49^{b}	0.06 ^{b}	13.55 ^{a}
3.	C.Nichi	169 ^{a}	60.30 ^{d}	1.03 ^{a}	0.60 ^{a}	0.09 ^{a}	12.00 ^{a}
4.	Pure Mysore	145 ^{c}	62.00 ^{d}	0.88 ^{bc}	0.55 ^{b}	0.05^{b}	11.53 ^{ab}
5.	TN White	145 ^{c}	65.30 ^{b}	0.74 ^{d}	0.50 ^{b}	0.05 ^{b}	9.33 ^{b}
	SEd	2.4328	0.7038	0.0529	0.0213	0.0077	1.1299
	CD (P=0.05)	5.2016	1.6216	0.0846	0.0454	0.0258	2.1647

In a column, means followed by same letter (s) are not significantly different (P=0.05).

Table 3. Effect of solvent extracts of botanicals on the larval mortality (%) and survival (%) of multivoltine breeds of *B. mori* exposed to BmNPV (KRI 1 strain).

Sl. No.	Treatments	Multivoltine breeds (larval mortality %)			Multivoltine breeds (survival %)		
		Nistari	C.Nichi	TN White	Nistari	C.Nichi	TN White
1.	*P. corylifolia*	35.65 $^{b}{}_{B}$	29.00 $^{b}{}_{A}$	34.00$^{a}{}_{A}$	63.00 $^{b}{}_{A}$	69.70 $^{b}{}_{A}$	66.88 $^{b}{}_{A}$
2.	*P. amboinicus*	34.60 $^{b}{}_{B}$	30.10 $^{b}{}_{A}$	33.00 $^{a}{}_{B}$	65.00 $^{b}{}_{B}$	70.50 $^{b}{}_{A}$	68.20 $^{b}{}_{A}$
3.	Treated	60.00 $^{c}{}_{A}$	59.00 $^{c}{}_{B}$	52.00 $^{c}{}_{C}$	37.00 $^{c}{}_{C}$	45.00 $^{c}{}_{B}$	55.00 $^{c}{}_{A}$
4.	Untreated	0.00 $^{a}{}_{A}$	0.00 $^{a}{}_{B}$	0.00 $^{a}{}_{C}$	100.00 $^{a}{}_{A}$	100.00 $^{a}{}_{A}$	100.00 $^{a}{}_{A}$
5.	Mean	36.8986	30.2154	30.1125	66.4800	70.1150	71.9611
	Sed	0.7507	0.4473	0.7291	2.1003	2.0183	2.1194
	CD (P=0.05)	1.8872	1.7231	2.6451	4.6434	4.1184	4.2291

Means followed by a common small letter in a column and capital letter (lower case) in a row are not statistically different by DMRT (P = 0.05).

doi: 10.5958/2582-2683.2023.00021.7

par with each other for the above treatments. No significant difference was observed between breeds for untreated control. C. Nichi recorded the highest larval weight of 0.78 g followed by TN White (0.67 g) and Nistari (0.56 g) for the untreated control (Table 4).

The cocoon weight in treatments, *P. amboinicus,* and *P. corylifolia* was the highest in Nistari being 0.80 g and 0.77 g, respectively, followed by both C. Nichi (0.69 g and 0.69 g) and TN White (0.66 g and 0.65 g) and both were on par with each other for the above treatments. No significant difference in cocoon weight was observed between breeds for untreated control. In the case of treated control, Nistari recorded the highest cocoon weight of 0.55 g, followed by C. Nichi (0.49 g) and TN White (0.54 g) (Table 5).

The shell weight in breeds C. Nichi and Nistari in *P. corylifolia* was 0.08g, 0.08g and in *P. amboinicus* it was 0.07 g and 0.09 g, respectively. There was no significant difference in shell weight between breeds for the untreated control. Nistari (0.05g) and C. Nichi (0.05 g) were on par followed by TN White (0.05 g) (Table 5).

The shell ratio in *P. corylifolia*, TN White (13.20 %), and C. Nichi (12.90%) were on par, followed by Nistari (10.50 %). In *P. amboinicus*, TN White recorded the highest shell ratio of 12.50 per cent followed by C. Nichi (12.00 %) and Nistari (11.50 %). No significant difference between breeds was observed for untreated control. In treated, C. Nichi recorded the highest shell ratio of 10.43 per cent followed by Nistari (9.50 %) (Table 6). Dandin *et al.* (2005) also reported that traditional races such as TN White are low in productivity and are suited for rearing under fluctuating temperature and poor environmental conditions. Studies conducted on the efficacy of botanicals against multivoltine breeds, *viz*., Nistari, C.Nichi and TN White infected with BmNPV revealed that *P.amboinicus* recorded significantly lesser mortality of 34.60 per cent, 30.10 per cent and 33.00 per cent respectively and was on par with *P.corylifolia* (35.60 %, 29.00 %, 34.00 %) against the mortality of 6000 per cent (Nistari), 59.00 per cent (C.Nichi) and 52.00 per cent in TN White) respectively. Several species of plants have been identified as effective antimicrobial agents (Shanmugasundaram *et al*., 1987; Marieorie, 1999; Dahanukar *et al*., 2000; Fiori *et al*., 2000). Botanicals serve as an environment-friendly management approach against diseases. In the present study, administration of botanicals, *viz*., *P. amboinicus* and *P. corylifolia* to multivoltine breeds recorded higher economic parameters, *viz*., larval weight, cocoon weight, shell weight, and shell ratio compared to treated control. The present result

Table 4. Effect of solvent extracts of botanicals on the larval duration (h.) and larval weight (g) of multivoltine breeds of *B. mori* exposed to BmNPV (KRI 1 strain).

Sl. No.	Treatments	Multivoltine breeds larval duration (h)			Multivoltine larval weight (g)		
		Nistari	C.Nichi	TN White	Nistari	C.Nichi	TN White
1.	*P. corylifolia*	162 $^{b}_{C}$	141 $^{a}_{A}$	148 $^{a}_{B}$	1.00 $^{a}_{B}$	1.10$^{a}_{A}$	1.00 $^{b}_{B}$
2.	*P. amboinicus*	166 $^{b}_{C}$	142 $^{a}_{A}$	149 $^{a}_{B}$	0.99 $^{a}_{B}$	1.07 $^{a}_{A}$	0.97 $^{b}_{B}$
3.	Treated	158 $^{a}_{B}$	141 $^{a}_{A}$	153 $^{a}_{B}$	0.56 $^{b}_{C}$	0.78 $^{b}_{A}$	0.67 $^{c}_{B}$
4.	Un treated	155 $^{a}_{A}$	149 $^{a}_{A}$	150 $^{a}_{A}$	1.03 $^{a}_{A}$	1.05 $^{a}_{A}$	1.04 $^{a}_{A}$
5.	Mean	155.74	145.25	150.74	0.8950	0.9775	0.9050
	SEd	1.1598	3.7992	2.5050	0.0063	0.0184	0.0172
	CD (P=0.05)	2.8237	9.3023	6.0908	0.0229	0.0448	0.0417

Means followed by a common small letter in a column and capital letter (lower case) in a row are not statistically different by DMRT (P = 0.05).

Table 5. Effect of solvent extracts of botanicals on the cocoon weight (g) and shell weight (%) of multivoltine breeds of *B. mori* exposed to BmNPV (KRI 1 strain).

Sl. No.	Treatments	Multivoltine breeds cocoon weight (g)			Multivoltine breeds shell weight (%)		
		Nistari	C.Nichi	TN white	Nistari	C.Nichi	TN white
1.	*P. corylifolia*	077 $^{b}_{A}$	0.69 $^{b}_{B}$	0.66$^{b}_{B}$	0.08 $^{c}_{A}$	0.08 $^{b}_{A}$	0.07 $^{b}_{B}$
2.	*P. amboinicus*	0.80 $^{b}_{A}$	0.69 $^{b}_{B}$	0.65$^{b}_{B}$	0.09 $^{b}_{A}$	0.07 $^{c}_{A}$	0.06 $^{c}_{B}$
3.	Treated	0.55 $^{c}_{A}$	0.49 $^{c}_{B}$	0.54 $^{c}_{B}$	0.05 $^{d}_{A}$	0.05 $^{d}_{A}$	0.05 $^{d}_{B}$
4.	Un treated	0.89 $^{a}_{A}$	0.89 $^{a}_{A}$	0.88 $^{a}_{A}$	0.10 $^{a}_{A}$	0.09 $^{a}_{A}$	0.09 $^{a}_{A}$
5.	Mean	0.7011	0.6577	0.6626	0.0850	0.0860	0.0700
	SEd	0.0156	0.0020	0.0099	0.0012	0.0013	0.0013
	CD (P=0.05)	0.0380	0.0047	0.0230	0.0055	0.0062	0.0054

Means followed by a common small letter in a column and capital letter (lower case) in a row are not statistically different by DMRT (P = 0.05).

doi: 10.5958/2582-2683.2023.00021.7

Table 6. Effect of solvent extracts of botanicals on the shell ratio (%) of multivoltine breeds of *B. mori* exposed to BmNPV (KRI 1 strain).

Sl. No	Treatments	Multivoltine breeds shell ratio (%)		
		Nistari	C.Nichi	TN White
1.	*P. corylifolia*	$10.50^{b}{}_{B}$	$12.90\ ^{a}{}_{B}$	$13.20\ ^{a}{}_{A}$
2.	*P. amboinicus*	$11.50\ ^{b}{}_{C}$	$12.00\ ^{b}{}_{B}$	$12.50\ ^{b}{}_{A}$
3.	Treated	$9.50\ ^{c}{}_{B}$	$10.45\ ^{d}{}_{A}$	$7.80\ ^{d}{}_{C}$
4.	Untreated	$10.60^{a}{}_{A}$	$10.27\ ^{a}{}_{A}$	$10.55^{c}{}_{A}$
5.	Mean	10.9076	11.4225	11.5325
	SEd	0.1731	0.1894	0.2257
	CD (P=0.05)	0.4066	0.4980	0.5478

Means followed by a common small letter in a column and capital letter (lower case) in a row are not statistically different by DMRT (P = 0.05).

on enhancing economic parameters on the administration of botanicals collaborates with Rajashekhar gouda (1991), who reported the growth-promoting effect of plant products. Application of petroleum ether extracts of *P. corylifolia* and *T. terrestris* increased the larval weight, cocoon weight, shell weight and shell ratio of *B. mori*.

CONCLUSION

It was concluded that the virulence of BmNPV KRI-1 strain tested against of multevi-line *Bombyse mori* showed high mortal of 75.03 per cent in Pure Mysore followed by 73.66 per cent in Tamil Nadu white. The experiment on efficacy of botanicals revealed that *P. amboinicus* recorded significantly lesset mortality of 34.60, 30.10 and 33.0 per cent which was almost on par at 35.65, 29.00 and 34.0 per cent in Nitsari, C. Nichi and T.N while, respectively.

ACKNOWLEDGEMENTS

Sincere thanks to the Students Advisory Committee, Department of Agricultural Entomology, The Dean, Adhiyamaan College of Agriculture and Research (Affiliated to Tamil Nadu Agriculture University Coimbatore-03), Athimugam-635105, Krishnagiri District, Tamil Nadu for providing necessary facilities and support for fulfilled research work.

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Online Manuscript No. 97159 recived on 14.3.2022
Manuscript accepted for publication on 19.11.2022

Journal of Eco-friendly Agriculture **18**(1): 130-135. 2023
doi: 10.5958/2582-2683.2023.00022.9

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Eco-friendly management of aphids in lucerne

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ABSTRACT

Among the eco friendly management practices for the management of aphids, the neem formulation (3000 ppm) recorded the maximum green fodder and seed yield. The next best treatments were *Vitex negundo* (Leaf juice extract) and garlic + chilli kerosene extract. Among entomopathogens, the *Lecanicillium lecanii* (@ 1×10^8 cfu g^{-1}) was effective at 7 DAS and up to 14 DAS. It also recorded the highest green fodder yield, dry matter yield as well seed yield and was found safer to natural enemies and pollinators. Although the standard check, Acetamiprid 20 SP was found superior in managing the aphids, it was found unsafe to natural enemies and pollinators.

Keywords: *Medicago sativa, Acyrthosiphon pisum,* lucerne, aphid, ecofriendly, management

Livestock industry continues to reveal a helpful impact on rural people in India by improving their income, employment and consumption and acting as a latent tool in alleviating rural poverty. Forages are the main input of our livestock industry. While any increase in the land area under forage crop is restricted due to competition between grain and cash crop, there is a huge scope for better agronomic practices to amplify forage productivity with superior quality. *Lucerne or alfalfa (Medicago sativa* L.) is one of the oldest cultivated fodder crops in the world. It is cultivated over an area of thirty five million hectares in the world. Its forage yield is 5-75m t ha^{-1} annually with 8-12 cuttings while seed yield is 186-280 kg ha^{-1} annually. It fixes 83 to 594 kg N per hectares (Aganga, 2003). In India, lucerne is third most important forage crop (Anonymous, 2011). It attracts considerable number of insects, like lucerne beetle, aphids, armyworms and the potato homopteran, that reduces lucerne yield significantly mostly with the second cutting when the weather is warmest. The cowpea aphid (*Aphis craccivora*) and pea aphid (*Acyrthosiphon pisum*) are the major insect pests causing significant losses in quality and quantity of green forage as well as the seed yield of lucerne especially in southern India. Numerous management components are found successful in managing the aphids, but biological control agents are gaining importance because of their safer to environment as well as soil and human health hazards (Kulkarni and Vinod Kumar, 2020).

Not much work seems to have been done on sucking pests infesting lucerne in Karnataka, although it is a major green leguminous nutritive fodder there. Since, it is fodder with frequent cutting systems; highly persisting insecticides are not suitable. With the progression of technology to maximize fodder production, the importance has now shifted from mere identification of biocontrol agent to their utilization as part of IPM for the pest control in lucerne. Emphasis is on the use of biocontrol agents, plants extracts and insecticides which are safe and environmentally harmless. Keeping this in view, an experiment on developing an appropriate, effective and eco-friendly pest management methodologies to manage aphids infestation in lucerne was taken up.

MATERIAL AND METHODS

Lucerne crop was raised during *rabi* season of 2020-21 by following the recommended package of practices. There were 10 treatments in the experiment. Each treatment was replicated thrice. Each cropped area was divided into 30 plots of 3 × 4 m with a buffer zone of 1 × 1 m between replication and also between treatments. Entomopathogenic fungi and other safe molecules were evaluated on lucerne at the farm of IGFRI, SRRS, Dharwad. Anand-2 variety of lucerne was used. The seeds were obtained from Indian Grassland and Fodder Research Institute (IGFRI), Southern Regional Research station (SRRS), Dharwad. All the biological control agents and vermiwash were obtained from the institute of organic farming, UAS, Dharwad. Ready formulations of entomopathogenic fungi were collected from Institute of Organic Farming, UAS, Dharwad. The details of insecticides used for experiment with their common name, formulation, and source are given in Table 1.

For the preparation of chilli-garlic kerosene extract, 100 g chilli and 100 g garlic were grinded to paste and soaked in 100 ml kerosene overnight. The liquid was extracted using

filter and added with 60 g of soap solution. All the ingredients were mixed thoroughly. Plant extract of *Vitex negundo* was prepared by crushing 100 g plant part with 100 ml of water. The extract was filtered through fine muslin cloth, and then the volume was made up to 200 ml by adding water to filtered extract. This product was at 50 per cent concentration and the desired concentration was prepared by adding distilled water.

The lucerne crop was raised as per standard package of practices at ICAR-IGFRI, SRRS, Dharwad as per following details:

Statistical design	: Randomized Block Design
Number of treatments	: 10
Number of replications	: 3
Plot size	: 3 × 4 m
Sowing season	: rabi, 2020-21
Variety	: Anand 2
Seed rate	: 10 kg/ha
Spacing	: 30 × 10 cm

Table 1: Treatment details

Tr. No.	Treatment details	Dose r^{-1}
T1	*Lecanicillium lecanii* (1 × 108 cfu ml^{-1})	2 gm l^{-1}
T2	*Beauveria bassiana* (1 × 108 cfu ml^{-1})	2 gm l^{-1}
T3	*Metarhizium anisopliae* (1 × 108 cfu ml^{-1})	2 gm l^{-1}
T4	Vermiwash	5-10 per cent
T5	Vitex negundo (leaf juice)	5 per cent
T6	Neem formulation (3000 ppm)	5 ml l^{-1}
T7	Chilli + garlic extract	5 per cent + 2.5 per cent aqueous)
T8	Cow urine	5 ml l^{-1}
T9	Acetamiprid 20 SP	1 g l^{-1}
T10	Untreated control	-

The treatments were applied as soon as infestation of aphids was observed on lucerne crop. Count of aphid population before the initiation of the treatments and after 1, 3, 7 and 14 days of sowing was recorded on 10 cm of tiller $plant^{-1}$ on five tagged plants. Need based treatments were imposed and two rounds of sprays were taken.

The amount of insecticides required for preparing the spray solution was calculated for each insecticide. At the time of preparing spray emulsion, the measured quantity of insecticide was mixed with little water. Then, the solution was poured in the bucket containing desired quantity of water. It was thoroughly stirred with the help of wooden stick. Spray solutions were applied with the help of a knapsack sprayer at evening. All the ten plots of each replication were treated at a time avoiding the drifts of spray fluid on neighboring plots. Care was taken to wash the spray pump with water thoroughly well before using other insecticide.

The data were transformed in to " n+ 0.5 values where "n" is number of insects and subjected to statistical analysis (Snedecor and Cocharan, 1967). Later, the data were fed to Randamized block design (RBD) in OPSAT software and Ducan's multiple range test (DMRT) was done in wasp software for the analysis and ANOVA Critical difference (CD) was calculated at p = 0.05 to identify the best treatment.

RESULTS AND DISCUSSION

The data presented in Table 2 and 3 showed that the pre counts of aphid population on lucerne were non-significant. Differences between the treatments were not that evident after first day of spray. However, there was a visible difference between treatments after three days of spray. Amongst all the treatments, neem formulation 3000 ppm @ 3 ml l^{-1} (103.53 aphids 10 cm^{-1} of tillers) was superior over all the treatments and followed by *V. negundo* leaf juice @ 5 per cent (218.33 aphids 10 cm^{-1} of tillers), garlic + chilli kerosene extract @ 5 +2.5 per cent (241.60 aphids 10 cm^{-1} of tillers). Entomopathogens *L. lecanii* 1×10^8 cfu g^{-1} @ 2g l^{-1}, *B. bassiana* 1×10^8 cfu g^{-1} @ 2g l^{-1}, *M. anisopliae* 1×10^8 cfu g^{-1} @ 2g l^{-1}, vermiwah @ 10 per cent and cow urine @ 5 per cent were on par with untreated check. Acetamiprid 20SP @ 0.2 g l^{-1} was considerably better over other treatments (26.33 aphids 10 cm^{-1} of tillers). Same trend was continued after 7 days of spray. However, entomopthogens, *L. lecanii* 1×10^8 cfu g^{-1} @ 2g l^{-1} (237.67 aphids 10 cm^{-1} of tillers) and *B. bassiana* 1×10^8 cfu g^{-1} @ 2g l^{-1} (316.87 aphids 10 cm^{-1} of tillers) found on par with *V. negundo* (Leaf juice) @ 5 per cent and garlic + chilli kerosene extract with 314.67 $aphids^{-1}$ to 10 cm^{-1} of tillers and 324.67 aphids 10 cm^{-1} of tillers, respectively. The remaining treatments *M. anisopliae* 1×10^8 cfu g^{-1} @ 2g l^{-1}, vermiwash @ 10 per cent and cow urine @ 5 per cent were at par with control. Acetamiprid 20 SP @ 0.2 g l^{-1} was significantly superior over rest of the treatments in reducing the aphid population (28.67 aphids 10 cm^{-1} of tillers). Similar trend continued even after 14 days of spray.

During second round of spray observations were taken on aphids count on 3, 7 and 14 days after spray. Treatment differences were not significant after first day of spray. After three days the lowest aphid population was recorded in neem formulation 3000 ppm @ 3 ml l^{-1} (100.00 aphids 10 cm^{-1} of tillers). Next best treatment was *V. negundo* (199.00 aphids 10 cm^{-1} of tillers) which was followed by garlic + chilli kerosene extract @ 5 +2.5 per cent (195.53 aphids 10 cm^{-1} of

tillers). However, after 7 days of treatment imposition, enotopathogens started showing significant efficacy *L. lecanii* 1×10^8 cfu g^{-1} @ 2 g l^{-1} (186.67 aphids 10 cm^{-1} of tillers) and *B. bassiana* 1×10^8 cfu g^{-1} @ 2 g l^{-1} (240.00 aphids 10 cm^{-1} oftillers) were found on par with *V. negundo* (leaf juice) @ 5 per cent (200.00 aphids 10 cm^{-1} of tillers) and garlic + chilli kerosene extract (202.20 aphids 10 cm^{-1} of tillers), respectively. However, *M. anisopliae* 1×10^8 cfu g^{-1} @ 2g l^{-1}, vermiwash @ 10 per cent and cow urine @ 5 per cent were at par with control recording aphid population of 372.00, 371.00, and 376.67 and 380.00 aphids 10 cm^{-1} of tillers, respectively. Acetamiprid 20 SP @ 0.2 g l^{-1} treated plot recorded very low aphid population (28.33 aphids 10 cm^{-1} of tillers). Similar trend continued even after 14 days of spray (Table 3). All the eco-friendly management approaches recorded higher green fodder yield (GFY), dry fodder yield (DFY) and seed yield over untreated check Neem formulation recorded 40.27 t ha^{-1} GFY, 11 t ha^{-1} DFY and 181.47 seed yield ha^{-1} which were on par with the treatments like *L. lecanii* 1×10^8 cfu g^{-1}, *V. negundo* (leaf extract) and garlic + chilli + kerosene extract (Table 4). All the eco-friendly management approaches were found safer to natural enemies like predators as well pollinators during all the rounds of spray during both rounds (Table 5 & 6).

These results are supported by the findings of Mani *et al*. (1990) who reported 85.4 to 95.4 per cent mortality of safflower aphids with neem oil emulsion (0.25%). Bhatt *et al*. (2007) reported that the plots treated with NSKE 5 per cent exhibited least aphids' population of 30.75 and 21.40 per 5 cm terminal twig, one and five days after spraying, respectively. Neem contained azadiractin which acts as anti-feeding agent. Effectiveness of NSKE and neem oil was also confirmed by the findings of Jagdale *et al*., (1990), Uthamasamy and Gajendran (1992), Mallapur *et al*. (2001), Sathyan *et al*. (2015) and Jat *et al*. (2018). Belmain *et al*. (2001) and Ayyanar *et al*. (2017) reported that *V. negundo* recorded the maximum mortality against sucking pests *i.e.* cowpea aphids on cowpea. The chemical content present in *V. negundo* plant had the capability to cause aphid mortality (Vatandoost and Moin 2001; Weathersbee and Mekenzie 2005). These findings are in agreement with the present study.

Nasseh and Furassy, (1992) showed that garlic clove contains 0.2-0.3 per cent allicin or alliin, diallyl disulphide and diallyl trisulphide, a principal larvicide component that proved a powerful insecticide and chilli contains capsaicin which acted as antifeeding and repulsion agent against

Table 2: Evaluation of eco-friendly approaches for the management of aphids in lucerne during first spray

Treatments		Dose	Pre-count	Average number of aphids 10 cm^{-1} of tillers			
				1DAS	3DAS	7DAS	14 DAS
T_1	*Lecanicillium lecanii* 1×10^8 cfu g^{-1}	2g l^{-1}	463.33 (21.54)	450.00 (21.22^a)	448.73 (21.20^a)	237.67 (15.43^b)	220.00 (14.85^b)
T_2	*Beauveria bassiana* 1×10^8 cfu g^{-1}	2g l^{-1}	494.00 (22.24)	456.67 (21.38^a)	449.33 (21.21^a)	316.87 (17.81^{ab})	320.20 (17.91^{ab})
T_3	*Metarhizium anisopliae* 1×10^8 cfu g^{-1}	2g l^{-1}	473.33 (21.77)	446.00 (21.13^a)	448.00 (21.18^a)	446.00 (21.13^a)	447.33 (21.16^a)
T_4	Vermiwash	10 per cent	466.67 (21.61)	441.53 (21.02^a)	460.00 (21.46^a)	445.13 (21.11^a)	445.13 (21.11^a)
T_5	*Vitex negundo* (Leaf juice)	5 per cent	463.33 (21.54)	236.00 (15.39^b)	218.33 (14.79^b)	314.67 (17.75^{ab})	325.33 (18.05^{ab})
T_6	Neem formulation 300 ppm	3 ml l^{-1}	480.00 (21.92)	136.53 (11.71^c)	103.53 (10.20^c)	110.33 (10.53^c)	105.00 (10.27^c)
T_7	Garlic + chilli kerosene extract	5+2.5 per cent	483.33 (22.00)	293.33 (17.14^b)	241.60 (15.56^b)	324.67 (18.03^{ab})	328.00 (18.12^{ab})
T_8	Cow urine	5 per cent	466.00 (21.60)	460.67 (21.47^a)	421.00 (20.53^a)	449.00 (21.20^a)	450.00 (21.22^a)
T_9	Acetamiprid 20 SP	0.2 g l^{-1}	470.33 (21.70)	46.67 (6.87^d)	26.33 (5.18^d)	28.67 (5.40^d)	26.67 (5.21^d)
T_{10}	Control	-	480.67 (21.94)	480.00 (21.92^a)	480.00 (21.92^a)	479.67 (21.91^a)	479.67 (21.91^a)
	S.Em (±)	**-**	**1.56**	**1.21**	**1.22**	**1.82**	**1.64**
	CD at 5%	**-**	**NS**	**3.62**	**3.66**	**5.45**	**4.92**
	CV (%)	**-**	**12.52**	**11.71**	**12.33**	**18.79**	**17.00**

DAS = Days after spray # Values in the parentheses are $\sqrt{x+0.5}$ transformed values used for statistical analysis.
Means followed by same alphabet in the same column do not differ significantly by DMRT

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00022.9

aphids. Allin is a sulfoxide that is a natural form of fresh garlic. It is also a derivative of amino acid cysteine. When fresh garlic is chopped or crushed the enzyme alliinase converts into allicin. Steam distilled garlic oil proved as more strong insecticide. Mode of action as well as the fungicidal and insecticidal properties of garlic is credited partly due to the enzyme inhibition.

L. lecanii attack nymphs and adults and sticks to the leaf underside by means of filamentous mycelium. Usually aphids were present more on undersides of leaves so it was effective against aphids, as reported by Nunez (2008). Several research workers have reported the efficiency of *L. lecanii* against aphids (Singh, 2003; Safavi *et al.,* 2002; Tambe 2009). Alate (2012) reported excellent control of lucerne aphids through combination of *L. lecanii* and *M. anisopliae*.

Table 3: Evaluation of eco-friendly approaches for the management of aphids in lucerne during second spray

Treatments		Dose	Average number of aphids 10 cm^{-1} of tillers			
			1 DAS	3 DAS	7 DAS	14 DAS
T_1	*Lecanicillium lecanii* 1×10^8 cfu g^{-1}	2g l^{-1}	380.00 (19.51^a)	373.33 (19.33^a)	186.67 (13.68^b)	191.67 (13.86^{bc})
T_2	*Beauveria bassiana* 1×10^8 cfu g^{-1}	2g l^{-1}	380.67 (19.52^a)	370.67 (19.27^a)	240.00 (15.51^b)	228.33 (15.13^b)
T_3	*Metarhizium anisopliae* 1×10^8 cfu g^{-1}	2g l^{-1}	382.00 (19.56^a)	372.00 (19.30^a)	372.00 (19.30^a)	385.53 (19.64^a)
T_4	Vermiwash	10 per cent	381.00 (19.53^a)	371.00 (19.21^a)	371.00 (19.27^a)	384.33 (19.62^a)
T_5	*Vitex negundo* (Leaf juice)	5 per cent	195.67 (14.01^b)	199.00 (14.12^b)	200.00 (14.16^b)	206.67 (14.39^b)
T_6	Neem formulation 300 ppm	3 ml l^{-1}	103.33 (10.19^c)	100.00 (10.02^c)	101.67 (10.11^c)	108.33 (10.43^c)
T_7	Garlic + chilli kerosene extract	5+2.5 per cent	198.87 (14.12^b)	195.53 (14.00^b)	202.20 (14.24^b)	210.53 (14.53^b)
T_8	Cow urine	5 per cent	380.00 (19.51^a)	370.00 (19.25^a)	376.67 (19.42^a)	380.00 (19.51^a)
T_9	Acetamiprid 20 SP	0.2g l^{-1}	30.00 (5.52^d)	28.33 (5.37^d)	28.33 (5.37^d)	41.67 (6.49^d)
T_{10}	Control	-	386.67 (19.68^a)	380.00 (19.51^a)	380.00 (19.51^a)	386.97 (19.68^a)
	S.Em (±)	-	**1.35**	**1.40**	**1.23**	**1.23**
	CD at 5%	-	**4.04**	**4.19**	**3.70**	**3.68**
	CV (%)	-	**14.90**	**15.62**	**14.34**	**13.99**

DAS = Days after spray # Values in the parentheses are $\sqrt{x+0.5}$ transformed values used for statistical analysis.
Means followed by same alphabet in the same column do not differ significantly by DMRT

Table 4: Impact of eco-friendly management on yield of lucerne crop

Treatments		Green fodder yield (t ha^{-1})	Dry fodder yield (t ha^{-1})	Seed yield (kg ha^{-1})
T_1	*Lecanicillium lecanii* 1×10^8 cfu g^{-1}	40.53	10.60	174.07
T_2	*Beauveria bassiana* 1×10^8 cfu g^{-1}	30.20	9.33	164.27
T_3	*Metarhizium anisopliae* 1×10^8 cfu g^{-1}	33.47	9.40	164.20
T_4	Vermiwash	33.73	9.20	162.60
T_5	*Vitex negundo* (leaf extract)	38.60	10.67	174.07
T_6	Neem formulation 300 ppm	40.27	11.00	181.47
T_7	Garlic + chilli + kerosene extract	39.13	10.87	174.33
T_8	Cow urine	33.07	9.20	162.00
T_9	Acetamiprid 20 SP	46.40	12.80	200.53
T_{10}	Control	30.67	8.13	159.27
	S.Em (±)	2.65	0.69	3.23
	CD at 5%	7.93	2.06	10.80
	CV (%)	12.56	11.75	11.18

doi: 10.5958/2582-2683.2023.00022.9

Table 5: Effect of eco-friendly approaches on coccinellids in lucerne during second spray

	Treatments	Dose	Average number of coccinellids/tillers			
			1 DAS	3 DAS	7 DAS	14 DAS
T_1	*Lecanicillium lecanii* 1×10^8 cfu g^{-1}	2 g l^{-1}	6.87 (2.71^a)	2.07 (1.60^c)	3.27 (1.94^{abc})	3.13 (1.69^a)
T_2	*Beauveria bassiana* 1×10^8 cfu g^{-1}	2 g l^{-1}	6.00 (2.55^a)	2.73 (1.80^{bc})	3.40 (1.97^{abc})	3.07 (1.62^a)
T_3	*Metarrhizium anisopliae* 1×10^8 cfu g^{-1}	2 g l^{-1}	6.17 (2.58^a)	2.53 (1.74^{bc})	2.73 (1.80^{bc})	3.87 (1.76^a)
T_4	Vermiwash	10 per cent	6.07 (2.56^a)	2.93 (1.85^{bc})	4.20 (2.17^{ab})	3.87 (1.62^a)
T_5	*Vitex nigundo* (leaf juice)	5 per cent	5.67 (2.48^a)	3.33 (1.96^{bc})	2.67 (1.78^c)	3.33 (1.60^a)
T_6	Neem formulation 300 ppm	3ml l^{-1}	5.67 (2.48^a)	3.33 (1.96^{bc})	3.40 (1.97^{abc})	3.67 (1.62^a)
T_7	Garlic + chilli kerosene extract	5+2.5 per cent	6.00 (2.55^a)	2.87 (1.83^{bc})	3.93 (2.11^{abc})	4.87 (1.60^a)
T_8	Cow urine	5%	6.27 (2.60^a)	3.60 (2.02^b)	3.20 (1.92^{abc})	3.87 (1.57^a)
T_9	Acetamiprid 20 SP	0.2 g l^{-1}	1.00 (1.2^b)	1.67 (1.47^d)	2.00 (1.58^d)	2.33 (1.68^b)
T_{10}	Control	-	6.40 (2.63^a)	5.47 (2.44^a)	4.33 (2.20^a)	3.53 (1.57^a)
	S.Em (±)	-	**0.19**	**0.12**	**0.13**	**0.13**
	CD at 5%	**-**	**0.58**	**0.36**	**0.38**	**0.40**
	CV (%)	-	**14.29**	**11.66**	**11.65**	**11.69**

DAS = Days after spray # Values in the parentheses are $\sqrt{x+0.5}$ transformed values used for statistical analysis.
Means followed by same alphabet in the same column do not differ significantly by DMRT

Table 6: Effect of eco-friendly approaches on pollinators in lucerne during first spray

	Treatments	Dose	Average number of pollinators/ tillers/min				
			Pre-count	1 DAS	3 DAS	7 DAS	14 DAS
T_1	*Lecanicillium lecanii* 1×10^8 cfu g^{-1}	2 g l^{-1}	6.33 (2.61)	5.17 (2.38^a)	5.00 (2.35^c)	5.00 (2.35^{abc})	4.67 (2.27^a)
T_2	*Beauveria bassiana* 1×10^8 cfu g^{-1}	2 g l^{-1}	6.00 (2.51)	5.00 (2.35^a)	5.67 (2.48^{bc})	5.73 (2.50^{abc})	3.67 (2.04^a)
T_3	*Metarhizium anisopliae* 1×10^8 cfu g^{-1}	2 g l^{-1}	6.67 (2.68)	5.67 (2.48^a)	5.27 (2.40^{bc})	5.67 (2.48^{bc})	5.33 (2.42^a)
T_4	Vermiwash	10 per cent	6.00 (2.55)	6.00 (2.55^a)	5.23 (2.39^{bc})	5.83 (2.52^{ab})	3.33 (1.96^a)
T_5	*Vitex negundo* (leaf juice)	5 per cent	6.33 (2.64)	6.00 (2.55^a)	5.27 (2.40^{bc})	6.00 (2.55^c)	3.33 (2.35^a)
T_6	Neem formulation 300 ppm	3ml l^{-1}	5.67 (2.48)	6.33 (2.61^a)	5.33 (2.42^{bc})	5.43 (2.44^{abc})	5.00 (2.20^a)
T_7	Garlic + chilli kerosene extract	5+2.5 per cent	6.60 (2.66)	5.00 (2.35^a)	5.00 (2.35^{bc})	5.83 (2.52^{abc})	4.33 (2.20^a)
T_8	Cow urine	5 per cent	6.33 (2.61)	5.33 (2.42^a)	5.17 (2.38^b)	5.73 (2.50^{abc})	3.67 (2.04^a)
T_9	Acetamiprid 20 SP	0.2 g l^{-1}	6.67 (2.36)	1.00 (1.22^b)	1.67 (1.47^d)	1.67 (1.47^d)	2.33 (1.68^b)
T_{10}	Control	-	6.93 (2.73)	5.00 (2.35^a)	5.83 (2.52^a)	6.00 (2.55^a)	5.00 (2.35^a)
	S.Em (±)	**-**	**0.25**	**0.15**	**0.18**	**0.16**	**0.15**
	CD at 5%	**-**	**NS**	**0.45**	**0.54**	**0.50**	**0.45**
	CV (%)	**-**	**17.08**	**11.69**	**14.03**	**12.73**	**11.26**

DAS = Days after spray # Values in the parentheses are $\sqrt{x+0.5}$ transformed values used for statistical analysis.
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Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00022.9

CONCLUSION

The study concludes that use of bio-contral measures and certain plant extracts, which are safer and environmentally harmless, could successfully be employed in migrating insect pests damage. Neem formulation (300 ppm) recorded the minimum number of aphids 10 cm^{-1} of tillers of lucerne and the maximum grain yield of 181.4 kg ha^{-1}.

ACKNOWLEDGEMENTS

Authors are thankful to the Director ICAR-IGFRI for providing research facilities to carry out this experiment.

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Online Manuscript No. 97054 recived on 8.3.2022
Manuscript accepted for publication on 12.11.2022

Journal of Eco-friendly Agriculture **18**(1): 136-139. 2023
doi: 10.5958/2582-2683.2023.00023.0

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Population dynamics of major insect pests of rice and the impact of climatic factors on their population

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ABSTRACT

The study on effect of different climatic factors on the abundance of major insect pest complex of rice, conducted under field conditions at BHU, revealed that the incidence of stem borer dead hearts (0.85%) was first noticed during the 32nd standard week (SMW) and culminated with a maximum of 9.37 per cent during the 48th SMW. The population of leaf folder was noticed during the 31st SMW with two peaks of 11.55 and 11.92 per cent of leaf damage during the 36th and 39th SMW, respectively. Incidence of ear head bugs noticed during the 31st SMW reached at its peak during the 36th SMW. Data showed a positive correlation with minimum temperature, rainfall and wind speed and a negative correlation with minimum relative humidity. In the current scenario of climate change, it is investable to know the population dynamics of a pest species to manage them in an economically effective manner.

Keywords: Climatic factors, ear head bug, leaf folder, population dynamics, stem borer

Rice is the world's most significant staple food crop with more than half of the world's population relying solely on it (FAO, 2004 & Heinrichs *et al*., 2017). More than 300 insect pests attack the rice crop, although around 20 of them cause economic damage (Pascal *et al*., 2006). Lepidopteron insects, such as the yellow stem borer, leaf folder and case worm are among those responsible for significant losses. The yellow stem borer (*Scirpophaga incertulus* Wlk) is a significant rice pest that attacks the crop at all stages. It is found in all Asian countries and can result in significant losses of up to 30 per cent. (Rahaman *et al*., 2014). The rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Pyralidae: Lepidoptera), is the most devastating leaf-feeding insect pest. Reductions in yield up to 80 per cent are possible in epidemic conditions. Areas receiving increased nitrogen fertilizer sprayes presents major problems. Under optimal environmental conditions, it causes 50-70 percent leaf loss and a 50 percent decline in rice yield (Kushwaha and Singh, 1984). Leaf folders are extremely common from October to January. *Leptocorisa acuta* (thumb.) (Hemiptera: Alydidae) is a serious rice insect pest in India, usually appearing before flowering and persisting till the milky stage. At the end of the rainy season, its population swelles to a dangerous levels. The yield may be reduced by as much as 30 per cent in severe situations of damage (Tiwari *et al*., 2014).

MATERIALS AND METHODS

The study was conducted under field conditions of BHU. In order to study the population building up of pests, a 100 m^2 bulk plot adjacent to the main experimental plots was raised. Moti variety of rice was cultivated in Randomized Block Design. Transplanting was performed with 25-day old seedlings by following the spacing of 20x15 cm.

Weekly observations on insect incidence were recorded from ten plants (hills) chosen randomly from the experimental plot from 30th standard week onward. The observations, continued until the crop was harvested, were recorded in the morning. Various abiotic weather data were recorded simultaneously from BHU's metrological observatory further analysis. Abiotic parameters such as maximum, minimum, and average temperatures, relative humidity in the morning and evening, precipitation, and maximum and minimum wind speeds were found to be associated to the seasonal prevalence of key rice pests. The data on per cent prevalent insect pests was used to determine their abundance in the crop by using the formula:

$$Per\ cent\ dead\ heart\ or\ white\ ears = \frac{no.of\ dead\ hearts\ (or)\ white\ ears}{(total\ no.of\ tillars)}\ X\ 100$$

$$Leaf\ folder\ damage\ (\%) = \frac{\text{No. of damaged leaves}}{\text{Total number of leaves}} \text{ X } 100$$

Whereas for ear head bug, the observations were recorded by sweeping the insect collection nets five times over each plot, and the number of nymphs and adults were counted.

RESULTS AND DISCUSSION

Stem borer

Incidence of stem borer in the rice crop was observed mainly during vegetative and panicle initiation stages and continued up to grain filling stage of the crop. During the crop growing period, minimum (21.10°C) and maximum (32.65°C) temperatures were noticed during the 49th and 30th standard weeks, respectively.

The first incidence of dead hearts (0.85%) was noticed during the 32nd standard week and culminated with a maximum of 9.37 per cent during September 4th week. It is in line with the results of Sabir *et al*., (2006) who also reported that dead hearts formation during September i.e., the vegetative stage of the rice crop. The pest showed a significantly negative correlation with minimum (r = -0.79) & maximum temperatures (r = -0.83) and non-significant negative correlation with rainfall (r = -0.12), minimum relative humidity (r = -0.34), minimum (r = -0.03) and maximum wind speed (r = -0.39).

The incidence of white ears (3.30%) was first noticed during 34th Standard week. It was in accordance with Sankpal, 2011. The pest showed a significantly positive correlation with minimum temperature (r = 0.52) (Kumar *et al*., 2001), rainfall (r = 0.87) (Adibourne, 2006), minimum wind speed (r = 0.59) and non-significantly positive correlation with maximum wind speed (r = 0.45). however, it showed a non-significantly negative correlation with maximum temperature (r = -0.17) (Adibourne, 2006) and a significantly negative correlation with minimum relative humidity (r = -0.57) (Kumar *et al*., 2001).

Leaf folder

Two peaks of leaf folder incidence were noticed during the 36th SMW & 39th SMW with 11.55% & 11.92% of leaf damage, respectively (Table 1). Similar results were reported by Hafeez *et al*., 2010; Khan and Ramamurthy, 2004. The population was the first time noticed during the 31st SMW and caused a moderate level of damage (10.03% of leaf damage) during 34th SMW. The data on leaf folder population showed a significantly positive correlation with minimum temperature (r = 0.61) (Chakrabarthy *et al*., 2011; Hafeez *et*

Table 1: Influence of abiotic factors on seasonal incidence of Major Insect Pests of rice during *Kharif* 2018-19

SE	Month and Date	Rainfall (mm day^{-1})	RH (%)	Temp Max °C	Temp Min °C	Temp Avg °C	WS range	WS Min	WS Max	Yellow stem borer		Leaf folder	Ear head bug
										DH (%)	WE (%)		
30	July, 21	8.12	67.07	35.13	27.36	32.65	4.17	1.21	5.61	0	0	0	0
31	July, 28	10.31	83.42	31.89	24.87	30.53	3.65	2.11	6.41	0	0	1.9	1.2
32	August, 4	35.16	88.53	30.26	24.54	29.73	3.14	2.54	1.53	0	0	2.8	1.4
33	August, 11	3.64	69.09	32.39	25.73	28.95	3.67	1.41	4.45	0	0	3.9	1.7
34	August, 18	4.51	75.81	31.11	26.10	27.16	3.01	1.31	4.46	0.85	0	4.4	2.10
35	August, 25	13.72	84.50	30.29	25.41	28.63	2.89	1.76	3.89	1.64	0	9.9	2.92
36	September, 1	8.97	86.31	30.19	25.74	28.17	2.58	0.83	3.92	3.10	0	10.2	3.15
37	September, 8	14.38	86.35	30.57	24.32	28.10	2.78	1.89	4.19	3.53	0	11.2	3.53
38	September, 15	0.096	85.21	31.53	24.53	27.78	2.49	2.35	4.47	7.10	0	11.55	3.43
39	September, 22	2.93	82.31	32.19	24.10	27.10	2.61	2.34	5.38	8.90	0	11.35	3.12
40	September, 29	0.03	80.58	32.47	24.01	27.19	2.69	1.53	3.99	9.12	0	10.6	3.19
41	October, 6	0	78.61	32.58	22.91	26.83	2.92	1.54	3.47	7.57	3.30	9.55	3.5
42	October, 13	0.09	73.59	30.15	21.54	26.84	2.47	0.89	3.15	6.15	4.21	8.3	2.01
43	October, 20	0.03	67.74	32.47	19.54	25.39	2.25	1.31	3.49	4.12	5.23	7.2	1.90
44	October, 27	0.17	58.68	32.89	18.91	24.28	2.51	0.61	2.90	2.13	5.66	5.4	1.73
45	November, 3	0	56.19	32.36	18.57	23.73	1.17	1.31	3.91	1.81	6.93	4	1.20
46	November, 10	0	54.43	30.42	17.54	22.10	2.31	0.99	3.73	0.21	6.52	7.2	1.05
47	November, 17	0	52.72	30.89	16.71	21.82	2.41	1.42	3.90	0	6.35	2	0.97
48	November, 24	0	46.54	29.40	15.73	21.81	1.79	0.89	3.94	0	6.11	1.55	0.94
49	December, 01	0	52.48	26.49	15.83	21.10	2.11	0.97	2.89	0	5.23	1.34	0.91

DH: Dead heart, WE: White ear, WS: Wind speed, RH: Relative humidity, Temp: Temperature, Avg: Average, Min: Minimum, Max: Maximum, SW: standard week

doi: 10.5958/2582-2683.2023.00023.0

Table 2: Correlation coefficient values of major insect pests of rice during *Kharif* 2018-19

Variables		Rainfall (mm day^{-1})	RH (%)	Temp Max ^{o}C	Temp Min ^{o}C	Temp Avg ^{o}C	WS range ms^{-1}	WS Min ms^{-1}	WS Max ms^{-1}
Yellow stem borer	**% of DH**	-0.128^{NS}	-0.349^{NS}	-0.832**	-0.794**	-0.385^{NS}	-0.558**	-0.030^{NS}	-0.391^{NS}
	% of WE	0.879**	-0.570**	-0.170^{NS}	0.524*	-0.598**	-0.342^{NS}	0.595**	0.459*
Leaf folder		0.785**	-0.350^{NS}	0.053^{NS}	0.619**	-0.417^{NS}	-0.165^{NS}	0.536*	0.529*
Ear head bug		0.323^{NS}	-0.087^{NS}	0.292^{NS}	0.444*	-0.085^{NS}	-0.146^{NS}	0.227^{NS}	-0.288^{NS}

*Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level, NS-Non-significant, DH: Dead heart, WE: White ear, WS: Wind speed, RH: Relative humidity, Temp: Temperature, Avg: Average, Min: Minimum, Max: Maximum

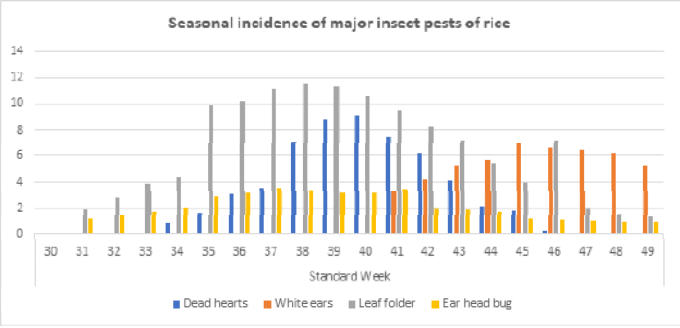

Figure 1: Seasonal incidence of major insect pests of rice during 2018-19

al., 2010) and non-significantly positive correlation with maximum temperature (r = 0.05) (Chakrabarthy *et al.*, 2011), rainfall (r = 0.78), minimum (r = 0.53) and maximum wind speeds (r = 0.52). It was also showed a non-significantly negative correlation with minimum relative humidity (r = -0.35) (Bhoopathi *et al.*, 2012) (Table 2, Fig. 1).

Ear head bug

Incidence of ear head bugs was noticed during the 31st SMW and it was similar to the findings of research conducted by Singh *et al.*, 2017. The peak population of ear head bugs was noticed during the 36th SMW and its population level oscillated over a period of two weeks 37th SMW & 38th SMW (Table 1). These results were in concurrence with Sharma *et al.*, 2004 and Sulagitti *et al.*, 2017 who reported that the ear head bug population reached a peak level during the milky stage of the crop. The population showed a significant positive correlation with minimum temperature (r = 0.44) and a non-significant positive correlation with maximum temperature (r = 0.29) (Bhatnagar and Saxena, 1999), rainfall (r = 0.32) (Sharma *et al*, 2004) & minimum wind speed (r = 0.22) apart from a non-significant negative correlation with relative humidity (r = -0.08) & maximum wind speed (r = 0.28) (Table 2, Fig. 1).

CONCLUSION

The population of stem borer, leaf folder, and ear head bugs was noticed after 20-25 (33rd SMW) and 15-30 (31st SMW) days after transplanting and reached to its peak level at 40th, 38th, and 37th SMW, respectively. Data showed a positive correlation with minimum temperature, rainfall & wind speed and a negative correlation with minimum relative humidity.

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Online Manuscript No. 97420 recived on 30.3.2022
Manuscript accepted for publication on 16.11.2022

Journal of Eco-friendly Agriculture **18**(1): 140-143. 2023
doi: 10.5958/2582-2683.2023.00024.2

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Predatory potential of reduviid bug, *Rhynocoris marginatus* (Fab.) against fruit borers of tomato

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ABSTRACT

The field experiment carried out to study the predatory potential of reduviid bug against tomato fruit borers during *Rabi* season of 2020-21 revealed that three augmentative releases of reduviid predator @5000 bugs ha^{-1} at 30, 50 and 70 days after transplanting of crop resulted in significant reduction of mean number of *H. armigera* and *S. litura* larvae plant^{-1} in predator released plot than against the control plot. The mean per cent fruit damage per plant was significantly lower at every picking in predator released plot over control plot. The fruit damage (12.92%) in predator released plot at second picking was significantly less than control plot (27.83%). The study showed that the release of predator played a significant role in reducing larval population and ultimately reducing tomato fruit damage.

Keywords: Reduviid predator, Field efficacy, Biological control, *Helicoverpa armigera*, *Spodoptera litura*

Tomato (*Solanum lycopersicum* L.) is an economically essential solanaceous vegetable crop which is adversely affected by fruit borers causing severe economic losses. Tomato fruit borer, *Helicoverpa armigera* (Hub.) causes losses up to 35 per cent of production (Paul, 2007; Wade *et al*., 2020; Anonymous, 2018) whereas, the polyphagous pest, tobacco caterpillar, *Spodoptera litura* (F.), cause substantial damage to foliage as well as 12 to 23 per cent fruit loss in tomato crop (Muthukumaran and Selvanarayanan, 2008; Patnaik, 1998). Development of a system of insect pest management by intelligently minimizing the pesticide load in the field crops is of the essence (Field and White, 2002). Reduviid bugs are significant predators of insect and their role in pest control had been revealed in past few decades. Reduviids kill the pests having soft cuticle by releasing the venomous saliva into the body of prey followed by sucking inner body content (Nagarajan, 2010; Smith, 1966; Sahayaraj *et al*., 2020). They are famous for killing more number of preys than they need to satiate their life, which makes them an effective biological control agent. *Rhynocoris marginatus* (Fab.) is found to have more preference against *S. litura* and *H. armigera* in groundnut fields (Ambrose and Claver, 1999b; Sahayaraj, 2002). Therefore, the study of biocontrol potential of locally observed species can be helpful to assess detailed knowledge for employing them in biological insect pest management (Ambrose, 1995; Grundy *et al*., 2000; Lakkundi, 1989). Thus, the present investigation was carried out in Konkan region to study the predatory potential of reduviid bug, *Rhynocoris marginatus* (Fab.) against tomato fruit borers under field conditions.

MATERIALS AND METHOD

The reduviid bug, *R. marginatus* was mass reared in laboratory on *Corcyra cephalonica* (Stainton.) as per the procedure discussed by NIPHM as well as Ambrose and Claver (1999a). Various life stages of predator were separately maintained and used for release in the field. Predatory potential against tomato fruit borers was studied in tomato field by making two equal blocks having 60 plants in each block. The crop was grown by following all suggested agronomic practices suggested by Dr. BSKKV, Dapoli, except any specific insect pest management practice. Reduviid bugs of third instar were released @5000 bugs ha^{-1} in one block (T1-Treated plot) at 30, 50 and 70 days after transplanting of the crop. The predator released block was covered from sides by a cloth fencing to avoid migration of predators from one block to another (T2- control plot). The observations were recorded as pre-count of number of larvae of tomato fruit borer, *H. armigera* (Hub.) and tobacco caterpillar, *S. litura* (Fab.) on each plant on the day of first release and subsequent post-counts were detailed on 3rd, 7th, 10th and 14th day after each release of predator. Similarly, the per cent fruit damage was recorded at 60, 70, 80, 90 and 100 DAT in both plots. Record was taken by maintaining randomization and the experimental data was subjected to analysis by two sample t-test.

RESULTS AND DISCUSSION

During the course of investigation, two major pests viz. tomato fruit borer (*H. armigera*) and tobacco caterpillar (*S. litura*) were witnessed to damage the tomato fruits in field

with their typical damaging habits. Therefore, the observations (Table 1) of mean number of larvae of *H. armigera* as well as *S. litura* per plant and per cent fruit damage by fruit borer were presented here under following subheadings.

Predatory potential of *R. marginatus* against tomato fruit borer (*H. armigera*) in under field conditions

The data (table 1) on mean number of tomato fruit borer (*H. armigera*) larvae plant^{-1} showed that, the incidence of tomato fruit borer (*H. armigera*) was found to commence from 37th day after transplanting (DAT). The mean number of larvae plant^{-1} did not vary significantly in both plots till 40th DAT (10 days after first released). However, the significantly lower number of larvae plant^{-1} in T1-predator released plot was observed first time at 44th DAT. At 53rd DAT, tomato fruit borer incidence in T1-predator released plot (0.75 larvae plant^{-1}) was significantly lower than in T2- control plot (1.4 larvae plant^{-1}) on the same day. However, at 57, 60, 64 and 73rd DAT, the suppressed pest incidence of tomato fruit borer was evidenced by lower mean population in T1-treated plot (0.65, 0.8, 0.55 and 0.45 larvae plant^{-1}) than in T2-control plot (1.7, 1.4, 1.4 and 1.95 larvae plant^{-1}), respectively. Among the all observations, the highest peak of tomato fruit borer in T2-control plot (2.4 larvae plant^{-1}) was observed on 77th DAT and in T1-predator released plot (0.8 larvae plant^{-1}) on 60th DAT. But, the previous trend of significantly lesser count of larvae was seen in T1-predator released plot at 77, 80 and 84 DAT and were recorded 0.4, 0.5 and 0.3 larvae plant^{-1} over T2-control plot (2.4, 1.8 and 2.05 larvae plant^{-1}), respectively. Thus, the cumulative effect of three releases resulted in reduction of mean number of larvae of tomato fruit borer (*H. armigera*) plant^{-1} in T1-predator released plot which is significantly superior over T2-control plot throughout the experiment.

Simillarly, Sahayaraj (1999) also revealed that the population of *H. armigera* and *S. litura* was reduced by 92.7 and 94.9 per cent, respectively due to release of *R. marginatus* in groundnut field. Sahayaraj and Martin (2003) revealed that the augmentative release of *R. marginatus* in groundnut field reduced the infestation of *S. litura*, *H. armigera*, aphids and grasshopper. Sahayaraj and Ravi (2007) released variety of life stages of *R. marginatus* and revealed that, lesser infestation of *H. armigera* was found in predator released plot than in the control plot. Similarly, the *S. litura* population also was found decreased in the predator released plot.

Predatory potential of *R. marginatus* against fruit borer (*S. litura*) in tomato field

The pre-count of *S. litura* larvae per plant recorded before first release of predator did not differ significantly in both plots. At 33rd DAT, the mean number of larvae per plant was recorded lesser in T1-predator released plot (0.45 nos.) than T2-control plot (0.9 nos.). There was no statistically significant difference among the treatments. However, the significantly lower number of larvae per plant was observed first time at 40th day after transplanting in T1-predator released plot (0.8 nos.). Further, the infestation was observed slightly increasing in T2-control plot (1.35 larvae plant^{-1}) and its peak was observed on 53rd DAT (1.85 larvae plant^{-1}). After 57th to 84th day, the T2-control plot witnessed proportionately similar pest infestation. But subsequently the T1-predator released plot witnessed lowering of pest population which was found significantly superior over

Table 1: Mean no. of tomato fruit borer (*H. armigera*) larvae plant^{-1} in tomato field

Days after transplanting	Mean no. of *H. armigera* larvae plant^{-1}		T value	Mean no. of *S. litura* larvae plant^{-1}		T value
	T1- Predator released Plot	T2- Control plot		T1- Predator released Plot	T2- Control plot	
Precount- 30	0	0	-	0.30	0.40	0.59
33	0	0	-	0.45	0.90	2.25
37	0.10	0.30	2.06	0.65	1.15	2.83*
40	0.70	1.15	2.09	0.80	1.35	3.32*
44	0.45	0.85	3.14*	0.75	1.60	4.19*
53	0.75	1.40	2.80*	0.85	1.85	7.30*
57	0.65	1.70	3.77*	0.55	1.40	8.50*
60	0.80	1.40	3.18*	0.65	1.35	3.61*
64	0.55	1.40	4.32*	0.55	1.45	6.93*
73	0.45	1.95	5.69*	0.40	1.45	5.94*
77	0.40	2.40	2.87*	0.25	1.50	4.88*
80	0.50	1.80	5.38*	0.40	1.40	6.41*
84	0.30	2.05	4.35*	0.20	1.55	5.27*

*Significant at 5% level (Table T value = 2.77)
Three release of predator were done at 30, 50 and 70 DAT.

doi: 10.5958/2582-2683.2023.00024.2

their respective observation in T2-control plot. The lowest mean (0.2 larvae plant^{-1}) was observed in T1-predator released plot over in T2-control plot (1.55 larvae plant^{-1}) at 84th DAT. The result revealed that, augmentative release of reduviid predator in tomato field reduced the mean number of fruit borer (*S. litura*) larvae per plant in T1-predator released plot than in T2-control plot throughout the experiment.

Per cent fruit damage by tomato fruit borers per plant

During the course of experiment, two major pests were witnessed damaging the fruits of tomato. The larvae of *H. armigera* damaged fruits by boring hole into it and the infested fruit become unpalatable. On the other hand, larvae of *S. litura* damaged tomato fruit from outer side. The per cent damage of fruits per plant altogether caused by both the pests is presented in Table 2.

In the first picking done at 60 DAT the fruit damage per plant in predator released plot was significantly lower (3.33%) than that of control plot (29.17%). The highest fruit damage in predator released plot (12.92%) recorded at second picking also was significantly less than control plot (27.83%). The consecutive three pickings done at 80, 90 and 100 DAT revealed lesser damaged fruits per plant in predator released plot than in the control plot. Hence, the predator released plot was found significantly superior for control of fruit damage over the control plot.

Table 2: Per cent fruit damage by tomato fruit borers

Days after transplanting	Mean per cent fruit damage plant^{-1}		T value
	T1- Predator released Plot	T2- Control plot	
60	3.33	29.17	8.44*
70	12.92	27.83	3.57*
80	7.25	32.92	3.79*
90	4.58	25.33	5.09*
100	2.25	27.50	7.46*

*Significant at 5% level (Table T value = 2.77)

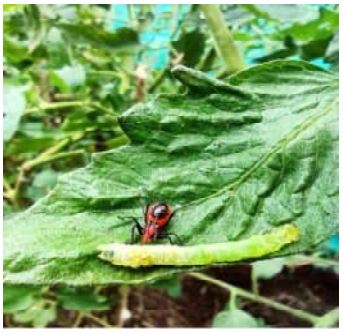

Fig. 1: *R. marginatus* nymph predating upon *H. armigera*

Fig. 2: *R. marginatus* nymph predating upon *S. litura* larva

Many previous studies on field efficiency of *R. marginatus* in pest management of various crops have recorded similar findings in different crops. Sahayaraj and Balasubramanian (2016) revealed that the population of *S. litura*, *A. craccivora*, *H. armigera* and *Pericallia ricini* was drastically reduced after release of predator in field. Femi Mohasina (2017) tested field efficacy of *R. marginatus* against pests of cowpea and observed that the predator was successfully able to reduce infestation of pod borer (*M. vitrata*) as well as aphids in field and *S. litura* in caged condition. The biocontrol potential of reduviid bug varies differentially according to their searching behaviour. Claver *et al*. (2002) suggested that, the potential is much related to the dispersal ability of bugs. Our present study demonstrated the lake results of release of predator in tomato field and it was proved to be a significant biocontrol agent.

CONCLUSION

The release of predator *R. marginatus* @ 5000 bugs ha^{-1} had played a significant role in reducing larval population and ultimately the fruit damage.

ACKNOWLEDGMENT

The authors are grateful to Department of Agricultural Entomology, Dr. BSKKV, Dapoli for providing technical help and guidance in the smooth conduct of experiment.

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Online Manuscript No. 97777 recived on 22.4.2022
Manuscript accepted for publication on 10.11.2022

Journal of Eco-friendly Agriculture **18**(1): 144-150. 2023
doi: 10.5958/2582-2683.2023.00025.4

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Mating disruption: An ecological step towards sustainable pest management

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ABSTRACT

The current method of semiochemical-based pest management that tries to diminish the reproductive success of the targeted insect pest is mating disruption. The overabundance of synthetic sex pheromones in the field confuses males, who are unable to locate its counterpart for mating, resulting in the collapse of insect-pest populations without the usage of pesticides. As mating disruption tools, several microencapsulated dispensers, manually applied dispensers (pheromone traps, PB rope, SPLAT) and high-emission dispensers are now available in the market. This approach has the potential to offer significant value to the long-term ecological pest management of several commercially significant insect pests, such as the rice yellow stem borer, Indian meal moth, American bollworm, shoot and fruit borer, fruit fly and pink bollworm, among others. This method not only decreases pesticide use but also aids in the preservation of insect pests' natural enemies. Consequently, this innovative technology may assist to address the gaps in current IPM programmes for sustainable pest management.

Key words: Mating disruption, behavioural control, semiochemicals, sex pheromones, PB Rope L, SPLAT

The green revolution is one of the most significant achievements for human beings that resulted in self-sufficiency in food production. For maintaining the status of self-sufficiency, humans have to protect their food because it suffers from various abiotic and biotic stress (such as diseases and insect-pest attacks) daily. Farmers are using too many insecticides to control these pests which leads to environmental pollution, insect resistance to insecticides, destruction of beneficial insects, and resurgence of secondary pests (Karuppuchamy and Venugopal, 2016). Nowadays, awareness about organic farming is increasing among people. In this era of organic farming, scientists have to work with nature and develop new technologies to drive sustainable agriculture to feed the world without destroying the planet. The IPM is a way that provides maximum yield with minimum losses. The IPM is an ecosystem-based strategy that focuses on the long-term prevention of pests or their damage through a combination of techniques such as biological control, habitat manipulation, modification of cultural practices and use of resistant varieties and insecticides in a way that minimizes environmental risks and health hazards. Among the different components of IPM, behavioural control mainly focuses on the manipulation of insect behaviour (*i.e.*, mating) by the use of semiochemicals (Dara, 2019).

Sex pheromones

A sex pheromone is a semiochemical released by an organism (usually female) to attract an individual of the opposite sex (male) of the same species for mating or other activity related to reproduction. Over 150 species of insects are known in which the females produce sex pheromones and about 50 species in which the males do so. It is useful in IPM for monitoring, mass trapping, and mating disruption (Bakthavatsalam, 2016). A pheromone communication system consists of three components: pheromone production, medium (air or water), and pheromone reception. The gland that produces pheromones is ectodermal in origin; it opens and releases its products outside the body. Therefore, it is called an exocrine gland. These glands can be present on any part of the body *i.e.* head, thorax, abdomen, legs, or wings-depending on the species. Pheromone receptors could be olfactory (smell) or gustatory (taste) sense organs and in the majority of insects it is olfactory type. The olfactory sensilla are present on the antennae. Pheromone molecules enter the sensillum through their numerous pores. These pores lead into the lumen of the sensillum which is filled with a fluid called the sensillum liquor. The pheromone molecules are possibly absorbed by the liquor and then move by diffusion to combine with the receptor site to initiate transduction (transformation of the stimuli into electrical impulses that reach the brain). When the male moth is activated by the

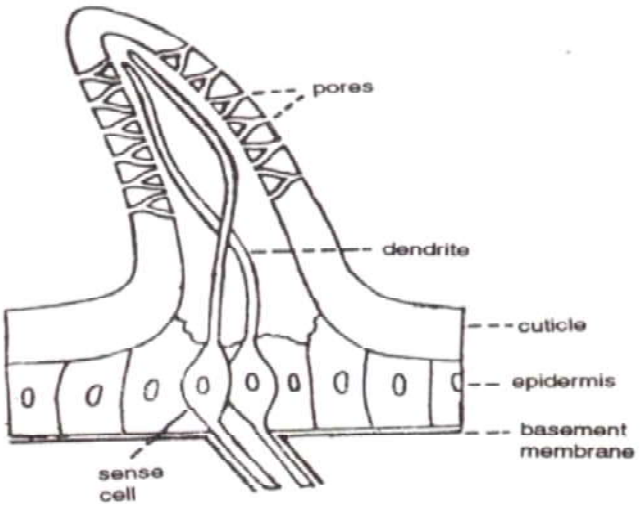

Fig. 1: Image of insect antennae and exocrine glands

pheromone, it simply flies upwind and moves towards the female (Ganai *et al*., 2017).

Mating disruption

Mating disruption is a pest management technique that aims to disrupt the chemical communication of organisms and interrupt normal mating behaviour by introducing synthetic sex pheromones, that confuse the individuals and disrupt mate localization, thus preventing mating thereby affecting the organism's chance of reproduction (Carde and Minks, 1995). Mating disruption was first discussed by the scientists of an institute in Bordeaux, France in 1974. This technology uses synthetically produced pheromones, typical blends of major chemical components; along with some minor components; which attempt to mimic the effects of naturally produced pheromones (Bakthavatsalam, 2016).

Synthetic sex pheromones

It involves a diverse group of volatile, predominantly lipid-like straight-chain aliphatic alcohols, esters, or aldehydes, but several terpenoid or polycyclic oxygenated structures have also been identified (Ganai *et al*., 2017). Over 2000 pheromonal compounds have been isolated and identified from volatile secretions of insect pests and disease vectors. The amount of the pheromone blend in traps generally ranges from 0.1 to 1000 mg, whereas mating disruption typically requires 50-200 g/ha of the pheromone to provide the necessary aerial concentration, typically 1-20 ng/m^3 for extended periods (Ujvary, 2010).

Mechanism of mating disruption

Ambient pheromone concentrations are sufficient to hide the trails of the calling female, so males become confused.

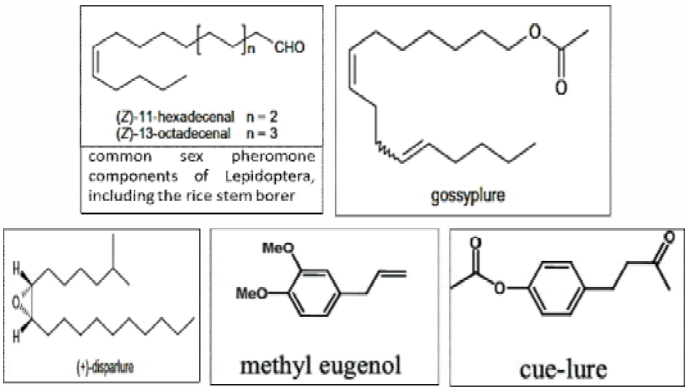

Fig. 2: Structure of some synthetic sex pheromones

When a receptor site is frequently activated by high concentrations of the synthetic pheromone, the resulting electrical signal weakens. The receptor site becomes unresponsive and the insect becomes navigationally blank. When the insect's central nervous system is flooded with signals from the receptor sites, it becomes habituated and no longer remains capable to provide the directed behaviour. The net result of confusion is that the male is unable to orient to any pheromone source and follow the upwind trail to a mate. So, also called as Male Confusing Technique (Mafra-Neto *et al*., 2014; Nandagopal *et al*., 2008).

Methods of pheromone application

1) Microencapsulated dispensers

It is a small droplet of pheromone encapsulated within polymer capsules. It controls pheromone release rate and ultraviolet (UV) degradation. It can be applied with standard spray equipment. The capsules are small in size (50 µm). Its field longevity ranges from a few days

Table 1. List of synthetic sex pheromones (Kotak and Patel, 2021)

Sl. No.	Pest	Crops	Pheromones
1	Melon fruit fly	Gourds	Cue lure [4 P hydroxy phenyl)-2 butanone acetate]
2	Oriental fruit fly	Fruit crops	Methyl eugenol [4-allyl-1,2-imethoxybenzone]
3	American bollworm	Chick pea, tomato, chilli, okra, pigeon pea, groundnut, cabbage	(Z)-11- hexadecenal and (Z)-9-hexadecenal
4	Armyworm	Castor, tobacco, cabbage, groundnut	(Z,E)-9,11-tetradecadienyl acetate
5	Spotted bollworm	Cotton, okra	(Z)-11-hexadecaienal-10, 12-Hexadecen-l-ol
6	Pink bollworm	Cotton	(Z,Z) and (Z,E)7,11-hexadecadienyl acetate (1:1)
7	Fall armyworm	Maize, sorghum, pearl millet, rice	(Z)-7-dodecadien-l-ol acetate
8	Diamondback moth	Cabbage, cauliflower, mustard	(Z)-11-hexadecenal, 11-hexadecanyl acetate and (Z) 11-hexadecenol (5:5:5)
9	Pink stem borer	Maize, sorghum, wheat	(Z)-11-hexadecenal
10	Yellow stem borer	Rice	Hexadecenal (Z)-9 hexadecenal
11	Semilooper	Castor	9 Heneicosadiene, (Z,Z,Z)-3,6,9-Heneicosatriene
12	Shoot and fruit borer	Brinjal	(Z)-11-Hexadecenyl acetate, (E)-11 hexaden-l-ol

to slightly more than a week (Sarfaraz *et al*., 2006; Ganai *et al*., 2017).

2) Hand applied dispensers

It is hollow plastic fibers (capillaries), polymer spirals, and "twist ties" (also known as tube-type, ropes, or clips) that consist of pheromone-filled plastic tubes with field longevity ranging from 60 to 140 days. It requires only a single application during the crop season There are three sub-types: (A) Pheromone traps (B) PB Rope L (C) SPLAT (Sarfaraz *et al*., 2006; Ganai *et al*., 2017).

(A) Pheromone traps

A pheromone-impregnated lure is encased in a conventional trap such as a bottle trap, delta trap, water-pan trap, funnel trap *etc.* Important sex pheromones which are useful: Disparlure (gypsy moth), gossyplure (pink bollworm), grandlure (cotton grey weevil), looplure (cabbage looper), helilure (American bollworm), litlure (Spodoptera) *etc.*

(B) PB Rope L

It is a sex pheromone dispenser of pink bollworms containing ZZ/ZE-7, 11-hexadecadienyl acetate in two plastic tubes of 2-3 mm diameter and 20 cm long, which are attached together by both ends. Pheromone is released gradually into the environment through the wall of the tube.

(C) SPLAT (Specialized Pheromone and Lure Application Technology)

It is a paste or cream-like consistency substance. It provides controlled release and sustainability. The most basic SPLAT applicator can be a stick, spatula, or knife. More advanced manual applicators include syringes, grease guns, and caulking guns. Its field longevity ranges from 2 weeks to 6 months.

3) High emission dispensers

It exploits a false trail followed by the release of large amounts of pheromone from a few point sources to avoid habituation caused by evenly spaced dispensers. For this purpose, metered systems such as micro sprayers have been developed which use a pressurized aerosol that can dispenses metered puffs of pheromone at fixed time intervals. It requires high-dose pheromone dispensers (Sarfaraz *et al*., 2006; Ganai *et al*., 2017).

Mating disruption (MD) in different crop pest management

Cereal crops

Cereals are an important source of carbohydrates and staple food for Indian people which are attacked by some devastating pests. Rice yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) is one of the most economically important biotic constraints to rice production throughout the Asian region. A commercially-available, hand-applied PVC resin formulation of the sex pheromone of the striped rice stem borer, *Chilo suppressalis* (Walker) was used to control *S. incertulas*, in a 20 ha MD trial in West Bengal in 1992. The results showed that the level of white ear damage in the pheromone-treated plot was significantly lower than that recorded in other treatment plots and that the relative percentage of the *S. incertulas* larvae found in the region changed from 88 per cent in the farmers' practice plot to 65 per cent in the pheromone-treated plot (Cork and Basu, 1996). Similarly, field trials were conducted at three locations in Warangal district-Andhra Pradesh, to evaluate the effectiveness of Auto confusion technology (Exo sex pheromone pellets-512 mg/ha) for management of YSB with farmers practice or mass trapping (20 funnel traps/ha). Auto confusion technique registered lower white ear incidence than mass trapping and farmers' practice. Auto confusion technique was found to be eco-friendly by harbouring a larger number of predator populations (Prasad *et al*., 2016).

doi: 10.5958/2582-2683.2023.00025.4

Pulse crops

Pulses are the major source of protein for vegetarians but they are very prone to storage insect pests. In dried bean storage and processing structure, the impact of MD and aerosol space treatment using synergized pyrethrins on Indian meal moth, *Plodia interpunctella* (Hubner) was experimented with using a high-volume aerosol timed release dispenser (Z,E)-9,12-tetradecyldienyl acetate (Z9,E12-14:Ac) with 1.9 mg/day/100 m^3 releasing rate for mating disruption. The biological effects of MD were compared between areas treated with mating disruption, aerosol space treatments, and an untreated part of the facility. The ability of males to orient to a pheromone source, to mate with calling females, and the fertility of resident females was examined using pheromone traps, sentinel females, and oviposition bait cups, respectively. Compared to an untreated area, males in pheromone traps and female mating were greatly reduced in both the aerosol space treatment and MD treatment areas. After the second week of the study, *P. interpunctella* progeny was recovered from the untreated area and the aerosol space treatment area but not from the MD area (Burks *et al*., 2011).

The MD technique was used in pea fields, for five years period to control pea moth, *Cydia nigricana* (Fab.) by assessing male attraction to monitor traps, pod infestation, and larval age structure in pheromone-treated and untreated grain. Cellulose pheromone dispensers were manually attached to the top shoots of pea plants and released 540 mg/ha/day synthetic pheromone (E8, E10-dodecadien-1-yl acetate). The dispensers had a half-life of about 30 days. Although male attraction to pheromone monitoring traps was largely suppressed at the edges and within MD fields (Saucke *et al*., 2014).

The use of synthetic pheromone lures has been developed as a more environmentally friendly alternative for its control. The potential of the pheromone components as an MD tool under laboratory and small-scale field conditions has been evaluated by identifying effective blends made out of single pheromone components or a different mix of them. The results from the laboratory experiment showed that insects challenged with the blend ratio of 1:1:1 had lower fecundity and egg exclusion. A small-scale caged field experiment also showed significant disruption of normal mating with the above-mentioned ratio, leading to lower flower and pod damage, and higher mung bean yield (Dhanyakumar *et al*., 2020).

Vegetable crops

Male Annihilation Techniques (MAT) for MD were evaluated against melon fruit fly, *Bactrocera cucurbitae* (Coquillett) using cue lure as pheromone application in bitter gourd. During two years experiment, different four places were selected to execute treatments such as area-wide control, farm-level control, area-wide + farm-level control, and no control. Among all levels, the least fruit infestation (18.76%) was found in cue lure block applied at area-wide + farm level control field. Maximum reduction in fruit infestation over no control (25.58%) was also found in the same treatment (Sisodiya, 2007).

The effectiveness of MD to control the tomato leaf miner, *Tuta absoluta* (Meyrick), in greenhouse tomato was evaluated in four trials carried out in winter–spring and summer–winter growing seasons in Southwestern Sardinia (Italy). Pheromone dispensers loaded with 60 mg of the natural blend of the major and minor sex pheromone component (9:1) were applied in greenhouses at a rate of 1000 dispensers/ha (60 g a.i./ha). Male captures in monitoring pheromone traps, percentage of tomato plants infested by *T. absoluta,* and damage on leaves and fruits were monitored weekly and compared in disrupted and untreated (control) greenhouses. It also reduced the damaged fruits by 62-89 per cent. The MD showed to be an efficient strategy to control the tomato leaf miner in the greenhouse and can be included in the overall integrated pest management programs in tomato (Cocco *et al*., 2012).

A field experiment was conducted at Raichur, Karnataka using "Specialized Pheromone and Lure Application Technology" (SPLAT) with different treatments like water trap, sleeve trap, and delta sticky trap in comparison with farmer's practices against shoot and fruit borer of brinjal. SPLAT was applied at two different doses of 250 and 500 g per acre. Both the SPLAT applications gave the least percent shoot and fruit damage as compared to others. SPLAT @ 500 g/acre was found the best and gave the least percent damage (Shridhara, 2018).

The diamondback moth (DBM), *Plutella xylostella* (Linnaeus) is the most destructive insect pest of cruciferous crops, particularly cabbage, broccoli, and cauliflower. An attempt was made to evaluate the EAG responses of DBM male antennae to different doses of synthetic sex pheromone blend (Z-11-hexadecenal, Z-11-hexadecenyl acetate, and Z-11-hexadecenol). The treatments were not statistically significant with increasing doses of 2% solution of standard formulation and control. The EAG response was initially increased with the increase in dose from 20 µL (0.841±0.67) to 30 µL (0.861±0.67) and then decreased from 40 µL (0.486±0.23) onwards. It showed that higher doses can cause desensitization in males and reduced mating (Topagi *et al*., 2018).

The shoot and fruit borer, *Leucinodes orbonalis* (Guenee)

a dreaded pest of brinjal showed the least percent shoot (1.06%) and fruit (0.98%) damage with minimum moth catches (3.86 moths/trap) which was found significant in Sawaj MDP @ 1200 g/ha treated plot over farmer's practices plot (insecticides treated) which proved the effectiveness of Sawaj MDP technology against brinjal shoot and fruit borer during 2016-18 at Junagadh, Gujarat (Anonymous, 2020).

Tomato fruit borer, *Helicoverpa armigera* (Hubner) is the most devastating pest of processing tomato in Italy. During a study of two years, geostatistical analysis of trap catches was shown to estimate the efficacy of the MD technique through the representation of the spatial pattern of captures. Lower fruit damage was recorded in mating disruption than in the untreated control plots, with variable efficacy depending on season and sampling date. Mating disruption showed higher efficacy than standard IPM in controlling *H. armigera* infestation (Burigo *et al*., 2020).

Fruit crops

The apple trees were heavily infested with the oriental fruit moth, *Grapholita molesta* (Busck). To manage this pest, the efficacy of mating disruption using Isomate-M 100 pheromone dispensers and a formulation of microencapsulated sprayable pheromone was compared with conventional insecticides in large plot studies in Henderson County, North Carolina during 2000 and 2001. Pheromone trap catches were significantly reduced in mating disruption blocks compared with conventional and abandoned orchards. Isomate-M 100 provided excellent trap shutdown and was significantly more effective than sprayable pheromone formulations. Fruit damage by oriental fruit moth larvae was very low (<1%) in mating disruption blocks and was generally lower than in conventional and untreated blocks. Mating disruption proved to be an alternative to organophosphate insecticides for managing oriental fruit moth populations in North Carolina apple orchards (Kovanci *et al*., 2005).

The mating disruption technique for control of the European vine moth, *Lobesia botrana* (Denis & Shiffermuller) was tested in 2003 and 2004. Comparison between plots treated with (a) *L. botrana* mating disruption pheromone, (b) *L. botrana* and *Cryptoblabes gnidiella* (Milliere) mating disruption pheromones, and (c) control plots (pesticide treated) was carried in wine grapes. A significant difference in the number of clusters infested with the developmental stages of the moths was seen between pheromone-treated plots and controls, while no such difference was observed between plots treated with one versus two pheromones. There was less than 1% male catches/trap/night observed in *L. botrana*-treated plots as compared to others (Harari *et al*., 2007).

An area-wide mating disruption trial was conducted in Mezzocorona vineyards during 1992 to 2001. Insecticides do not achieve a long-term pest population decrease. In contrast, an observation shared by working with pheromone-based control is that continuous long-term use decreased population levels of target species. This is attributable to a recovering fauna of beneficials, and to an increased efficacy of pheromones at low population densities when communication distance between sexes is increasing. (Witzgall *et al*., 2010).

The series of experiments on codling moth (*Cydia pomonella* L.) control using the mating disruption method were conducted in three experimental orchards during 2006-2010 growing seasons. The efficacy of commercial pheromone Ecodian CP, in comparison to pesticides Calypso 480 SC and Appeal 04 PA, gave the least percentage of damaged fruits, number of caterpillars, and number of moths caught in pheromone traps. The mating disruption method significantly reduced the level of apple damage during each experiment (Pluciennik, 2013).

The fruit fly least percent fruit damage (3.74%) with minimum fruitfly catches (2.86 flies/trap) were recorded in mango orchards treated with Sawaj MDP @ 1200 g/ha treated plot over farmers' practices plot (insecticides treated) which proved the effectiveness of Sawaj MDP technology against fruit fly of mango at Junagadh, Gujarat during 2017-18 (Anonymous, 2019).

The MD trials were conducted to evaluate the potential of citrophilous mealybug, *Pseudococcus calceolariae* (Maskell) to MD in an apple and a tangerine orchard during 2017-2020. Where two pheromone doses, 6.32 g/ha (2017-2018) and 9.45 g/ha (2019-2020) were tested. The intermediate season (2018-2019) was evaluated without pheromone renewal to study the persistence of the pheromone effect. In both orchards, in the first season, male captures were significantly lower in MD plots compared to control plots, with an MDI > 94% in the first month after pheromone deployment. During the second season, an average MDI of 80% was observed. In both orchards, the population by visual inspection and infested fruits were very low, without differences between MD and control plots which showed the potential use of mating disruption for the control of *P. calceolariae* (Ballesteros *et al*., 2021).

Cash crops

The SPLAT-PBW against PBW in *Bt* cotton ecosystem has rain fastness, easy to apply, long-lasting properties, and is cost-effective. To assess its feasibility in *Bt* cotton, dissipation studies were carried out by collecting filed samples of SPLAT-PBW dispensers at the weekly interval

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00025.4

and were subjected to gas chromatography analysis, wherein the results revealed that even by the end of the fifth week 40.36 percent of active ingredient i.e. pink bollworm pheromone ((ZZ/ZE) 7, 11- Hexadecadienly acetate) was left in the samples collected which, clearly indicates the slow release mechanism of SPLAT-PBW compared to other lures (Shrinivas *et al*., 2019a).

Cotton is heavily infested with pink bollworm, *Pectinophora gossypiella* (Saunders). An experiment was carried out during 2004-2006 at Anand, Gujarat against the pink bollworm for the first time introducing the PB Rope LTT, a pheromone dispenser. During two years experiment, 100, 150, and 180 PB Rope LTT/ha were compared with the control and results exhibited the minimum larval population (2.81 larva/80 green bolls) in 180 PB Rope LTT installed plots which showed the maximum mating disruption in the field (Anonymous, 2006).

The characteristic mating behaviour of the white grub beetle, *Dasylepida ishigakiensis* (Niijima & Kinoshita) a serious insect pest of sugarcane, has led to the identification of (R)-2-butanol (R2B) as a major sex pheromone component. Infestation can be effectively controlled by releasing either synthetic R2B or racemic 2-butanol (rac-2B) in the field to facilitate disruption of the sexual communication in the white grub beetle (Yasui *et al*., 2012).

To evaluate pheromone-based mating disruption technology with the help of mating disruption paste against pink bollworm, *P. gossypiella* in cotton on farmers' field of Jamnagar in Saurashtra region, a field trial was carried out during *kharif*, 2016. The results showed that the percentage of rosette flower (1.90%), percent green bolls (1.33%), percent open bolls (2.88%), percent locule damage (3.58%), and moth catches per trap (3.90 moths/trap) recorded in the treated plot was significantly least than the farmer's practices plot (Jethwa *et al*., 2018).

A wax-based formulation, "Specialized Pheromone and Lure Application Technology" (SPLAT) was evaluated at Raichur, Karnataka during *kharif* 2017 with different treatments like sleeve trap and delta sticky trap. The SPLAT was applied at three different doses of 500, 750, and 1250 g per acre, each treatment implemented in a one-acre area. The lowest rosette flowers (6.38%), green bolls (6.86%), and locule damage (12.28%) were found in SPLAT @ 500 g per acre compared to 16.01, 19.41 and 40.83 per cent in farmers' practice, respectively (Shrinivas *et al*., 2019b).

The effective reduction of pink bollworm in SPLAT pheromone treated block resulted in a reduction in green boll damage which contributed to higher seed cotton yield of 28.85 and 26.50 q/ha during 2018-19 and 2019-20, respectively with more net profit. Considering the yield advantage, SPLAT pink bollworm pheromone was found to be an economically viable and promising option for pink bollworm management in *Bt* cotton (Patil *et al*., 2022).

CONCLUSIONS

Mating disruption is a modern approach to semiochemical-based pest control, that aims to reduce the reproductive success of the target pest. The over-abundance of the synthetic sex pheromones in the field confuses males and they can't find females for mating which collapse the insect pest population without a single drop of insecticide. Nowadays different microencapsulated dispensers, hand-applied dispensers (pheromone traps, PB rope, SPLAT), and high-emission dispensers are available in the market as mating disruption tools. This technology has a huge potential to add value in long-term ecological pest management of many economically important insect pests *viz*., rice yellow stem borer, Indian meal moth, American bollworm, shoot, and fruit borer, fruit fly, pink bollworm, *etc*. Thus, this novel technology can help in filling the gaps in existing IPM programs for sustainable pest management.

ACKNOWLEDGEMENT

This manuscript is part of a minor doctoral seminar during Ph. D. programme. Hence, the authors are grateful to the Department of Agricultural Entomology, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand for providing the necessary facilities.

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Online Manuscript No. 100642 recived on 24.8.2022
Manuscript accepted for publication on 29.11.2022

Journal of Eco-friendly Agriculture **18**(1): 151-155. 2023
doi: 10.5958/2582-2683.2023.00026.6

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Field evaluation of different IPM modules against fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize

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ABSTRACT

Field trails on effectiveness of different IPM modules, including chemicals and cultural methods, against fall armyworm, *Spodoptera frugiperda* (J.E. Smith) infesting maize, conducted at University Research Farm, BCKV, Gayeshpur, West Bengal during 2021 and 2022, revealed significant reduction in larval population (60.75-56.12 per cent and 59.38-59.5 per cent) together with increased yield (57.33-50.67 q ha^{-1} and 58.17-51.50 q ha^{-1}) over control IPM in module comprising hybrid napier grass as border + chlorantraniliprole (10%) + lamdacyhalothrin (5% ZC) @ 45g a.i ha^{-1} at 3 weeks after sowing + indoxacarb 14.5 per cent SC @ 50g a.i ha^{-1} 5 weeks after sowing +lamdacyhalothrin 5 per cent EC @ 25g a.i ha^{-1}, 7 weeks after sowing. The module 6 comprising with spinetoram 11.7 per cent SC (30g a.i ha^{-1}) at 3 weeks after sowing and chlorantraniliprole 18.5SC (50g a.i ha^{-1}) at 5 weeks after sowing and emamectin benzoate 5 per cent SG (10g a.i ha^{-1}) at 7 weeks after sowing both the years of experimentation after sowing was found on par in respective parameters during 2021 and 2021, respectively. Two other modules, one with intercropping of coriander + spinosad 45 per cent SC @ 60g ai ha^{-1} at 3 weeks after sowing + azadirachtin 10,000ppm @ 5g a.i ha^{-1} at 5 weeks after sowing + chlorantraniliprole 18.5 per cent SC @ 50g a.i ha^{-1} at 7 weeks after sowing and the other comprising of chlorantraniliprole 18.5SC @ 50g a.i ha^{-1} three applications at 3, 5 and 7 WAS, were moderately effective in the management *S. trugiperda*.

Keywords: Fall armyworm, treatment modules, Maize, Napier grass, chlorantraniliprole, lamda cyhalothrin, spinetoram, emamectin benzoate

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a migratory insect pest infesting more than 353 plant species. Plants from families Poaceae (106), Asteraceae (31) and Fabaceae (31) are often attacked (Montezano *et al*., 2018). It is one of the most destructive pests of maize affecting annual crop productivity in tropical areas (Andrews, 1980; Marenco *et al*., 1992). It also damages over 80 other crops (Anonymous, 2019 and 2020b; De Groote *et al*., 2020). *S. frugiperda* infestation to maize crop threatens the food security of hundreds of millions of people since it serves as a most important staple grain for over 300 million people in most African countries. The pest is indigenous to North, Central, and South America's tropical and subtropical climates. Originally identified on the African continent in Nigeria in 2016 (Goergen *et al*., 2016), and by 2019, the pest spread throughout the Sub-Saharan Africa as well as South and Southeast Asia, leading to severe yield reductions (Rwomushana *et al*., 2018; Baudron *et al*., 2016).

Insecticides are generally used to manage this pest. Its ability to migrate over long distances and feed on a variety of hosts renders other control methods ineffective (Belay *et al*., 2012). Insecticides are most commonly used tool to manage fall armyworms in America and Africa (Hardke *et al*., 2011; Gutierrez-Moreno *et al*., 2019; Prasanna *et al*., 2018; Sisay *et al*., 2019). Many farmers expanded their use frequently and non-judiciously (Kassie *et al*., 2020; Tambo *et al*., 2020). Over-reliance on pesticides proved very problematic and hazardous because of potential environmental and health risks as well as the fall armyworm's extraordinary capacity to develop resistance (Zhu *et al*., 2015). More information on environment friendly alternatives to these dangerous synthetic pesticides are needed to enable farmers and governments to make educated choices. Cultural strategies, such as early planting, have been suggested as an effective management strategy (Prasanna *et al*., 2018). Maize and legume particularly the bean intercropping, may help in lessening FAW damage, particularly when beans are seeded earlier than the maize (Hailu *et al*., 2018). Push-pull techniques have significantly reduced FAW populations in several circumstances under African environments (Midega *et al*., 2018). Development of an efficient, low-risk, sustainable and economical management strategies and products thus becomes most important. The field effectiveness of

insecticides against the fall armyworm must also be determined to incorporate it with Integrated Pest Management techniques. Keeping this in view several insecticides including the biopesticides and cultural management strategies were evaluated.

MATERIALS AND METHODS

The field experiments were conducted in randomized block design (RBD) on maize crop at University Research Farm, BCKV, Gayeshpur, West Bengal during 2021 and 2022. The maize variety P 3396 (hybrid) was sown in *Rabi*, 2021 and *Pre-kharif*, 2022. The plot size was 5 m × 4 m and row to row and plant to plant distance was 50 × 30 cm. All the recommended agronomical practices were followed to maintain healthy crop. Eight treatment modules (Table 1) were taken with three replications. There spraying of the chemicals, the first 3 weeks after sowing (WAS) and remaining two at 14 days intervals were applied.

Visual observations on number of live larvae per plant was recorded 1 d before and 3, 7, 10, and 14 d after application of each treatment on ten randomly selected plants from each plot keeping aside the border rows. Marketable cob yield was recorded and expressed in quintal per hectare. The data was subjected to statistical analysis after suitable transformations.

Table 1. Treatment modules used in the management of *S. fugiperda* on maize

T1	Intercropping with coriander+ spinosad 45 per cent SC @ 60g ai ha^{-1} at 3WAS + azadirachtin 10,000ppm @ 5g a.i ha^{-1} at 5 WAS + chlorantraniliprole 18.5 per cent SC @ 50g a.i ha^{-1} at 7WAS
T2	Hybrid napier grass on border + intercropping with coriander + azadirachtin 10,000ppm @ 5g a.i ha^{-1} 3 sprays at 15 days interval from 3WAS
T3	Hybrid napier grass on border + (chlorantraniliprole 10 per cent + lamda cyhalothrin 5 per cent ZC) @ 45g a.i ha^{-1} at 3WAS + indoxacarb 14.5 per cent SC @ 50g a.i ha^{-1} 5 WAS + lamda cyhalothrin 5 per cent EC @ 25g a.i ha^{-1} at 7WAS
T4	Chlorantraniliprole 18.5SC @ 50g a.i ha^{-1} three applications at 3, 5 and 7 WAS
T5	*Bacillus thuringiensis* v. *kurstaki* with spore count of 2×10^6 cfu g^{-1} @ 1000g a.i ha^{-1} at 3WAS + chlorantraniliprole 18.5 per cent SC @ 50g a.i ha^{-1} at 5WAS + *B. thuringiensis* v. *kurstaki* with spore count of 2×10^6 cfu g^{-1} @ 1000g a.i ha^{-1} at 7 WAS + chlorantraniliprole 18.5 per cent SC @ 50g a.i ha^{-1}
T6	Spinetoram 11.7 per cent SC (30g a.i ha^{-1}) at 3WAS and chlorantraniliprole 18.5SC (50g a.i ha^{-1}) at 5WAS and emamectin benzoate 5 per cent SG (@ 10g a.i ha^{-1}) at 7WAS
T7	Hybrid napier grass on border +*B. thuringiensis* v. *kurstaki* 2×10^6 cfu g^{-1} @ 1000g a.i ha^{-1} at 3WAS and *Beauveria bassiana* 5×10^7 cfu g^{-1} @ 5g $litre^{-1}$ at 5WAS and *B. thuringiensis* v. *kurstaki* 2×10^6 cfu g^{-1} @ 1000g a.i ha^{-1} at 7WAS
T8	Control (untreated check)

RESULTS AND DISCUSSION

Field efficacy of different treatment schedules for management of fall armyworm

Rabi season (2021)

First, second, and third applications of all the pesticides significantly ($p< 0.05$) decreased the fall armyworm larvae at 3, 7, and 10 days after treatment compared to the control (Table 2).

The larval population per plant varied between 2.23 to 2.64 a day before spraying and were statistically non-significant ($P = 0.05$). Modules T6 and T3 recorded the lowest number of larvae (1.29 and 1.31, respectively) after the first spray and were statistically on par with each other. After the second spray, modules T3 (1.12 larvae $plant^{-1}$) and T6 (1.25 larvae $plant^{-1}$) had the lowest larval populations per plant, followed by module T4 with 1.53 larvae $plant^{-1}$. All of the treatment modules significantly decreased the number of fall armyworm larvae relative to the untreated check on all observation days following the third spray. Lowest number of larval population was observed in modules T1, T3 and T6 with 1.00 larvae $plant^{-1}$ followed by T4 (1.05 larvae $plant^{-1}$) which were significantly on par with each other. Module T7 of the treatment program had the lowest success rate in lowering the overall mean larval population (1.84 larvae $plant^{-1}$). With regard to yield, the module T6 recorded the highest cob yield (58.17 q ha^{-1}) and was significantly on par with T3 (57.33 q ha^{-1}) followed by T4 (53.83 q ha^{-1}), T1 (52.17 q ha^{-1}), respectively (Table 1).

Pre *kharif* season (2022)

The larval numbers per plant, a day before treatment, ranged from 2.15 to 2.69 in all treatments, and were non-significant ($P< 0.05$). After first spray, all the treated plots exhibited significantly lower number of larvae as compared to untreated check (Table 3). Module T6 and T3 showed lowest number of larvae 1.19 and 1.25 per plant, respectively, and were significantly on par with each other. After second spray, a similar trend in larval population was recorded, where modules T6 (1.18 larvae $plant^{-1}$) and T3 (1.35 larvae $plant^{-1}$) showed lowest number of larvae followed by modules T4 (1.44 larvae $plant^{-1}$) and T1 (1.47 larvae $plant^{-1}$). At 3, 7, 10 and 14 d after the third spray it was observed that all the treatment modules significantly reduced the fall armyworm larvae compared to the untreated check. Lowest larval population observed in module T6 (1.00 larvae $plant^{-1}$) followed by T3 (1.04 larvae $plant^{-1}$) and T4 (1.08 larvae/ plant) were significantly on par with each other. Module T7 was significantly different from the rest of the treatment modules and was least effective in reducing the overall mean

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00026.6

larval population (1.74 larvae plant^{-1}). In terms of cob yield, module T6 provided the highest grain yield (51.50 q ha^{-1}) and was significantly on par with module T3 (50.67 q ha^{-1}).

Development of an alternate management strategy is required since most of the farmers use toxic pesticides without wearing protective coverings. Besides, the broad-spectrum pesticide kill many non-targetted organism, particularly the natural enemies, (which may be regulating FAW (Davis *et al.*, 2018). In the current investigation, the results of both the plantings demonstrated that the fall armyworm larval densities were considerably lower at 3, 7, 10, and 14 days after application of treatments for the 3 sprays in modules T3 comprising of hybrid napier grass on border + (chlorantraniliprole 10 per cent + lamda cyhalothrin 5 per cent ZC) @ 45g a.i ha^{-1} at 3WAS + indoxacarb 14.5 per cent SC @ 50g a.i ha^{-1} 5 WAS + lamda cyhalothrin 5 per cent EC @ 25g a.i ha^{-1} at 7WAS and the module T6 comprising of spinetoram 11.7 per cent SC (30g a.i ha^{-1}) at 3WAS and chlorantraniliprole 18.5SC (50g a.i ha^{-1}) at 5WAS and emamectin benzoate 5 per cent SG (10g a.i ha^{-1} at 7WAS. Therefore, it was observed that bio-rational insecticides along with cultural methods (border cropping) provided effective control of FAW. The next best treatment modules were T4 (chlorantraniliprole 18.5SC @ 50g a.i ha^{-1} three applications at 2 weeks interval) and T1 (intercropping with coriander+ spinosad 45 per cent SC @ 60g ai ha^{-1} at 3WAS + azadirachtin 10,000ppm @ 5g a.i ha^{-1} at 5 WAS + chlorantraniliprole 18.5 per cent SC @ 50g a.i ha^{-1} at 7WAS) and significantly superior than untreated check. The modules T7 comprising of hybrid napier grass as border + *Bacillus thuringiensis* v. *kurstaki* 2×10^6 cfu g^{-1} @ 1000g a.i ha^{-1} at 3WAS and *B. bassiana* 5×10^7 cfu g^{-1} @ 5g litre^{-1} at 5WAS and *B. thuringiensis* v. *kurstaki* @ 1000g a.i ha^{-1} at 7WAS was least effective.

The findings of this study are in consonance with Roy *et al*., 2021, who found that the module containing emamectin benzoate+ novaluron (Brazide), spinetoram (delegate), and chlorantraniliprole+ lambda cyhalothrin (ampligo) had the highest cumulative efficacy (9.88 and 10.19 per cent plant damage) with a significantly higher yield (52.39 and 52.66 q ha^{-1}) in both the experimental seasons.

Chlorantraniliprole 18.5 SC, emamectin benzoate 5 SG, spinetoram 11.7 SC, flubendiamide 480 SC, indoxacarb 14.5 SC, lambda cyhalothrin 5 EC and novaluron 10 EC were the most effective insecticides, according to research on FAW control in maize by Deshmukh *et al.*, 2020. They also noticed that the increased effectiveness was associated with better grain production in comparision to the control. Hardke *et al.*

Table 2. Efficacy of different treatment modules against fall armyworm on rabi maize under field conditions, 2021.

	Number of FAW larvae plant^{-1}*																			
	1st spray						2nd spray					3rd spray								
Treatment	**1 DBS**	**3 DAS**	**7 DAS**	**10 DAS**	**14 DAS**	**Mean**	**3 DAS**	**7 DAS**	**10 DAS**	**14 DAS**	**Mean**	**3 DAS**	**7 DAS**	**10 DAS**	**14 DAS**	**Mean**	**Cummulative mean**	**Reduction over control (%)**	**Yield (q ha^{-1})**	**Increase in yield over control (%)**
T1	5.33	1.67	2.00	2.67	3.00	1.58	2.00	2.33	1.00	1.33	1.17	0.00	0.00	0.00	0.00	0.00	1.33	47.78	52.17	42.01
	(2.52)	(1.63)	(1.72)	(1.91)	(1.99)	(1.82)^{cd}	(1.72)	(1.82)	(1.38)	(1.52)	(1.62)^{b}	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)^{d}	(1.53)			
T2	4.67	2.00	1.70	3.67	4.67	2.50	1.67	1.33	2.00	1.33	1.58	1.33	1.00	0.67	1.33	1.09	1.88	42.23	47.20	28.40
	(2.37)	(1.73)	(1.61)	(2.16)	(2.38)	(1.99)^{bc}	(1.61)	(1.52)	(1.66)	(1.52)	(1.59)^{b}	(1.52)	(1.38)	(1.28)	(1.49)	(1.43^{bc}	(1.69)			
T3	4.68	0.67	0.70	0.67	1.00	0.58	0.67	0.33	0.00	0.00	0.83	0.00	0.00	0.00	0.00	0.00	0.33	60.75	57.33	56.06
	(2.38)	(1.28)	(1.28)	(1.24)	(1.38)	(1.31)^{e}	(1.28)	(1.14)	(1.00)	(1.00)	(1.12)^{d}	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)^{d}	(1.15)			
T4	4.00	1.33	1.70	1.67	1.67	1.42	1.67	1.67	1.00	1.33	1.08	0.00	0.00	0.33	0.00	0.08	1.04	51.54	53.83	46.50
	(2.23)	(1.52)	(1.63)	(1.63)	(1.63)	(1.60)^{d}	(1.63)	(1.58)	(1.38)	(1.47)	(1.53)^{bc}	(1.00)	(1.00)	(1.14)	(1.00)	(1.05)^{d}	(1.42)			
T5	5.67	2.33	2.70	4.33	4.00	3.12	3.33	1.00	1.33	1.33	2.25	0.67	1.00	1.00	0.67	0.83	1.96	41.30	51.33	39.73
	(2.57)	(1.82)	(1.88)	(2.31)	(2.23)	(2.08)^{bc}	(2.03)	(1.38)	(1.49)	(1.49)	(1.65)^{b}	(1.28)	(1.41)	(1.41)	(1.28)	(1.34)^{c}	(1.72)			
T6	5.00	0.67	0.30	0.33	1.33	0.42	0.33	0.67	0.67	0.67	0.42	0.00	0.00	0.00	0.00	0.00	0.42	59.38	58.17	58.25
	(2.44)	(1.28)	(1.14)	(1.14)	(1.52)	(1.29)^{e}	(1.14)	(1.28)	(1.28)	(1.28)	(1.25)^{cd}	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)^{d}	(1.19)			
T7	4.33	2.33	3.00	4.33	4.67	2.83	3.67	1.67	2.00	1.67	2.33	1.67	1.00	1.33	1.33	1.33	2.40	37.20	47.10	28.12
	(2.30)	(1.82)	(1.99)	(2.29)	(2.36)	(2.13)^{b}	(2.13)	(1.63)	(1.66)	(1.58)	(1.78)^{b}	(1.63)	(1.41)	(1.50)	(1.52)	(1.53)^{b}	(1.84)			
T8	6.00	6.67	7.00	9.00	7.67	7.42	8.00	7.33	8.67	9.67	7.08	8.00	6.00	6.67	5.33	6.50	7.58	-	36.80	-
	(2.64)	(2.75)	(2.89)	(3.15)	(2.94)	(2.94)^{a}	(3.00)	(2.89)	(3.11)	(3.25)	(3.07)^{a}	(3.00)	(2.64)	(2.80)	(2.52)	(2.77)^{a}	(2.93)			
C.D. (p=0.05)	N/A	0.37	0.46	0.48	0.45	0.25	0.55	0.49	0.61	0.56	0.30	0.24	0.27	0.25	0.32	0.14			1.89	
SE(m)	0.14	0.12	0.15	0.16	0.15	0.08	0.18	0.16	0.20	0.18	0.10	0.08	0.09	0.08	0.10	0.04			0.62	

*Mean of 10 plants/treatment

Values in the parentheses are square root transformed

NS – Non significant, DBS – Days before spraying, DAS- Days after spraying

In a column means followed by common alphabets are not significantly different by DMRT (P < 0.05)

doi: 10.5958/2582-2683.2023.00026.6

Table 3. Efficacy of different treatment modules against fall armyworm on pre-kharif maize under field conditions, 2022.

	Number of FAW larvae/plant																			
	1st spray						2nd spray					3rd spray								
Treatment	**1 DBS**	**3 DAS**	**7 DAS**	**10 DAS**	**14 DAS**	**Mean**	**3 DAS**	**7 DAS**	**10 DAS**	**14 DAS**	**Mean**	**3 DAS**	**7 DAS**	**10 DAS**	**14 DAS**	**Mean**	**Cummulative mean**	**Reduction over control (%)**	**Yield (q ha^{-1})**	**Increase in yield over control (%)**
T1	5.00	1.67	1.33	2.00	1.33	1.58	1.33	1.33	1.00	1.00	1.17	0.67	0.33	0.00	0.00	0.25	1.00	49.28	44.17	47.22
	(2.43)	(1.63)	(1.52)	(1.72)	(1.52)	(1.60)^{c}	(1.52)	(1.52)	(1.41)	(1.41)	(1.47)^{cd}	(1.28)	(1.14)	(1.00)	(1.00)	(1.11)^{c}	(1.41)			
T2	5.33	2.33	1.33	3.00	3.33	2.50	1.67	1.67	1.67	1.33	1.58	1.00	0.67	0.67	0.33	0.67	1.58	42.09	38.03	26.60
	(2.51)	(1.82)	(1.52)	(2.00)	(2.06)	(1.87)^{b}	(1.63)	(1.63)	(1.63)	(1.52)	(1.60)^{c}	(1.41)	(1.28)	(1.28)	(1.14)	(1.29)^{bc}	(1.61)			
T3	6.33	1.00	0.33	0.67	0.33	0.58	1.33	1.00	0.67	0.33	0.83	0.33	0.00	0.00	0.00	0.08	0.50	56.12	50.67	69.06
	(2.69)	(1.38)	(1.14)	(1.28)	(1.14)	(1.25)^{d}	(1.52)	(1.41)	(1.28)	(1.14)	(1.35)^{de}	(1.14)	(1.00)	(1.00)	(1.00)	(1.04)^{c}	(1.22)			
T4	3.67	2.00	1.67	1.00	1.00	1.42	1.33	1.33	1.00	0.67	1.08	0.33	0.33	0.00	0.00	0.16	0.89	50.72	49.50	65.14
	(2.15)	(1.72)	(1.63)	(1.41)	(1.38)	(1.55)^{c}	(1.52)	(1.52)	(1.41)	(1.28)	(1.44)^{cd}	(1.14)	(1.14)	(1.00)	(1.00)	(1.08)^{c}	(1.37)			
T5	5.00	2.33	2.67	4.33	3.33	3.17	3.00	2.33	2.00	1.67	2.25	0.67	0.67	0.33	0.00	0.42	1.94	40.07	45.83	55.47
	(2.43)	(1.82	(1.88	(2.31)	(2.08)	(2.04)^{b}	(1.99)	(1.82)	(1.73)	(1.63)	(1.80)^{b}	(1.28)	(1.28)	(1.14)	(1.00)	(1.18)^{c}	(1.72)			
T6	5.33	0.67	0.00	0.67	0.33	0.42	0.67	0.33	0.67	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.28	59.57	51.50	71.85
	(2.49)	(1.28)	(1.00)	(1.28)	(1.14)	(1.19)^{d}	(1.28)	(1.14)	(1.28)	(1.00)	(1.18)^{e}	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)^{c}	(1.13)			
T7	6.00	2.33	2.33	3.00	3.67	2.83	2..67	3.00	2.00	1.67	2.33	1.33	1.00	0.67	0.67	0.92	2.03	38.27	37.67	25.54
	(2.62)	(1.81)	(1.82)	(2.00)	(2.16)	(1.96)^{b}	(1.91)	(2.00)	(1.73)	(1.63)	(1.82)^{b}	(1.52)	(1.41)	(1.28)	(1.28)	(1.38)^{b}	(1.74)			
T8	5.67	7.00	7.00	8.33	7.33	7.42	6.67	7.33	7.67	6.67	7.08	6.33	6.00	5.33	5.00	5.66	6.72	-	30.00	-
	(2.58)	(2.83)	(2.83)	(3.05)	(2.88)	(2.90)^{a}	(2.77)	(2.89)	(2.94)	(2.77)	(2.84)^{a}	(2.70)	(2.64)	(2.52)	(2.45)	(2.58)^{a}	(2.78)			
C.D. (p= 0.05)	N/A	0.42	0.40	0.32	0.47	0.17	0.31	0.26	0.22	0.30	0.19	0.37	0.33	0.28	0.20	0.09			2.839	
SE(m)	0.11	0.14	0.13	0.10	0.15	0.05	0.10	0.09	0.07	0.10	0.06	0.12	0.11	0.09	0.07	0.03			0.927	

*Mean of 10 plants/treatment

Values in the parentheses are square root transformed

NS – Non significant, DBS – Days before spraying, DAS- Days after spraying

In a column means followed by common alphabets are not significantly different by DMRT ($P < 0.05$)

(2011) found that lambda cyhalothrin provided a 40 per cent reduction in insecticide-treated whorls in sorghum, and flubendiamide (0.098 kg ai ha^{-1}), novaluron (0.088 kg ai ha^{-1}) and chlorantraniliprole (0.0101 kg ai ha^{-1}) provided effective reductions in infestation (2.5, 5.0, and 2.5 per cent, respectively). According to Hailu *et al.* (2018), the infestation of FAW in traditional push-pull technology (PPT) systems and climate-smart PPT systems were 36 and 38 per cent, respectively, compared to maize mono-crop, where 95 per cent infestation was reported. In PPT fields, maize infestation by FAW was lower than in bean, soybean, and groundnut intercropped fields. Maize intercropping with leguminous crops showed less severe infestations than mono-cropped maize, and the effects were highly substantial.

In the current study, the insecticide treatments led to a decrease in larval infestation and increase in crop yields during both the planting seasons. One of the most crucial elements in maize protection from fall armyworm larva damage was excellent pesticide coverage of the plant's whorl. This study advances our knowledge of the effectiveness of cultural and bio-rational pesticides with distinctive modes of action in the battle against fall armyworm. Additionally, these modules provided efficient fall armyworm protection across the two distinct planting seasons, leading to a high yield. Despite variations in lethal activity speed, the majority of the insecticides tested in this study were efficient in suppressing FAW when applied along with cultural methods of control. This might be ascribed to regular pest reconnaissance and pesticide use in rotation or combination, which may have served to delay resistance development and hence kept the FAW population receptive to those insecticides. Further research into the biology of the pest and the phenology of the crop to establish the best timing and frequency of application of such pesticides along with other IPM methods under field circumstances might aid in achieving high levels of control.

CONCLUSION

It is concluded that the insect pest management module comparing hybrid napier grass as catch crop on border + chlorantranilipole 10 per cent + lamdaeghalothrin 5 per cent ZC @ 45 g a i ha^{-1} 3 weeks after sowing + indoxacarb 14.5 per cent SC @ 50 g a.i. ha^{-1} 5 weeks after sowing + lamdaeyhylothrin 5 per cent EC @ 25 g a i ha^{-1} after 7 weeks of sowing that brought about 60.75 and 56.12 per cent reduction in FAW larval population and 56.06 and 69.06 per cent increase in maize yield over control during 2021 and 2022, respectively and therefore can be employed as an economic and ecofriendly module for management of SWA.

ACKNOWLEDGMENTS

Authors are thankful to KVK, University Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur, West Bengal for the technical support.

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Online Manuscript No. 99287 recived on 29.7.2022
Manuscript accepted for publication on 11.11.2022

Journal of Eco-friendly Agriculture **18**(1): 156-160. 2023
doi: 10.5958/2582-2683.2023.00027.8

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Role of *Trichogramma chilonis* in the management of *Plutella xylostella* in Cabbage under Manipur condition

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ABSTRACT

The experiments conducted at Bio-control laboratory and Shade net house of Entomology department, College of Agriculture, CAU, Imphal during November- March, 2021-22 revealed that the optimum release dosage of *T. chilonis* on diamond/back moth (DBM) eggs in 100:5 host parasitoid ratio accounted for highest per cent parasitization (83.6%) as against lowest per cent parasitization (40.6%) at 100:1 host parasitoid ratio. The results also revealed that parasitoid emergence increased with the increase in parasitoid number and maximum adult emergence (98.6%) was observed from 100:20 ratio indicating emergence of more than one adult from a single egg. Two release of *T. chilonis* @ 5 adults plant^{-1} twice (2 and 5 days after DBM moth release) was found to be most effective treatment followed by combined application with malathion i.e. *T. chilonis* @ 5 adults plant^{-1} once + one spray of malathion (1 ml l^{-1}) against the pest recording lowest larval population of 4.6 and 6.55 per plant, respectively. The results indicated that *T. chilonis* is a very efficient parasitoid in suppression of cabbage DBM (*Plutella xylostella*) under Manipur condition and can be explored as a potential bio control agent against lepidopterous insect pests of agricultural importance.

Keywords: *Trichogramma chilonis*, *Corcyra cephalonica*, *Plutella xylostella*, developmental period, adult longevity, parasitism, emergence, temperature, malathion.

Diamondback moth (*Plutella xylostella* Linnaeus) is a major insect pest in more than 100 countries across the globe; it affects cruciferous plants, especially *Brassica oleracea* crops such as cabbage, cauliflower, broccoli, brussels sprout and turnip. DBM originated first from North America in 1854 (Saravaiya and Patel, 2005). Because of its ability to develop resistance to virtually all major groups of insecticides, including *Bacillus thuringiensis* Berliner (Bt), much attention has therefore been given to biological control using parasitoids.

Manipur, which is a small, landlocked, north-eastern states of India, the production share of vegetables out of the total fruit and vegetable crops production in the state has increased from about 26% in 2005-06 to about 40% in 2015-16, however it is still smaller compared to the national production share of vegetables. In terms of production, the percentage share of cabbage was 30.78% of the total vegetable production in Manipur (Devi, 2019). Due to unusual rainfall and uncontrolled pesticide application in Manipur many pests on tomato and cabbage have increased, developed resistance to pesticides and often there are secondary outbreak of pest (Ningthoujam and Singh, 2015).

Secondary pest outbreaks, pesticide resistance, more stringent pesticide regulation and concern about human health and environmental quality have renewed the interest in Integrated Pest Management programs that emphasize biological control. The biological control of pests is mainly based on the principle that an insect-pest is having the natural enemies such as predators, parasites and pathogens. Of the different bio-agents, the family Trichogrammatidae (Order: Hymenoptera) has few unbeaten biological control agents for agricultural and forest pests (Qu *et al*., 2020; Singh and Haldhar, 2020). Globally, *Trichogramma* spp. is a stronghold of augmentative biological control and is applied yearly to about 15 million hectares across 40 countries (Zang *et al*., 2021). *Trichogramma* are extremely tiny wasps of size <1mm belonging to the family Trichogrammatidae of the order Hymenoptera comprising of about 800 species belonging to 90 genera. With about 230 species described worldwide, *Trichogramma* is the largest genus in the family constituting over a quarter of the known genera in this family. The highest number of species recorded in USA (58) followed by India, Brazil, China and Russia (Jalali *et al*., 2016). In India, about 26 Trichogrammatids are recorded; out of which *Trichogramma chilonis* (Ishii), *Trichogramma japonicum* (Ashmead) and *Trichogramma acheae* Nagaraja and Nagarkatti are of significant importance. *T. chilonis* can be more effective when its parasitizing potential and searching ability is well adapted in the field.

MATERIALS AND METHODS

The cultures of *Plutella xylostella* and *Corcyra cephalonica* were maintained under laboratory condition on the natural diet. The rearing temperature was maintained at 28°C ± 2°C and 65 ± 5 per cent relative humidity at Bio-control Laboratory, Department of Entomology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India during the months of November to March, 2021-2022.

Corcyra cephalonica culture was maintained under the same laboratory conditions. For its maintenance 1 cubic centimetre of *Corcyra* eggs was sprinkled per wooden box containing 2.5 kg of sterilized broken maize (culture medium) and was mixed up thoroughly followed by covering the box with lid. The larvae fed on broken maize until it went for pupation. Moth started emerging within 45-50 days. These were collected inside the net by glass tubes and later transferred to egg laying chamber. The eggs laid inside the chamber were collected daily with brush and used for further experimental purpose.

The laboratory strain of *T. chilonis,* procured from ICAR-NBAIR, Bengaluru, were reared on *C. cephalonica* eggs. Around 0.5cc of eggs was used for a single card (1cc of eggs consists of around 16000-18000 eggs). The *corcyra* card was then inserted to a glass tube having *Trichogramma* adults for parasitization. *T. chilonis* adults usually emerged after 8-9 days and were provided with fresh cards for culture maintenance.

Mass rearing of *Plutella xylostella* to maintain DBM eggs for experiment, was done using its larvae and pupae collected from the cabbage fields. The larvae were reared on cabbage leaves. The emerged adults were released into 34 cm x 34cm x 26cm rearing cage (Miura & Kobayashi 1993) housing mustard plants in small pots for oviposition.

Twelve hour-old eggs of *P. xylostella* were utilized for estimating the *optimum* release doses of *T. chilonis*. The leaf bit containing 100 DBM eggs was placed in test tubes (20x4 cm) for parasitization. A fine streak of 50 per cent honey solution was provided as adult food in each test tube. The eggs in each test tube were exposed to five treatments T1 - one mated female of parasitoid (100:1), T2 - five females (100:5), T3 - ten females (100:10), T4 - fifteen females (100:15) and T5 - twenty females (100:20). Each treatment was replicated five times. The females were allowed to parasitize for 24 hours and the leaf bits with parasitized eggs was kept in fresh test tubes for further development and emergence. The experiment was conducted at 25±1°C temperature and 65±5 relative humidity.

The extent of parasitization of DBM eggs was determined by counting the black coloured eggs.

The number of parasitoids emerged out of total parasitized eggs were counted and per cent adult emergence determined. The time taken in from first day of parasitization to adult emergence in days was recorded to determine developmental time for the egg parasitoid. The adult longevity was worked out from their emergence till death in days.

Role of *T. chilonis* alone and in combination with malathion in management of *P. xylostella* on cabbage

For determining the role of *T. chilonis* alone and in combination with malathion in management of *P. xylostella* on cabbage, an experiment was conducted in a shade net house at College of Agriculture, Central Agricultural University, Iroisemba, Imphal, located at latitude 24° 4 μN, longitude 93° 56 μE and at an elevation of 790 meters above mean sea level. One month old seedlings of cabbage variety "Pride of India", procured from Central Farm, College of Agriculture, CAU, Imphal, was planted in the 60 earthen pots following all recommended agronomic practices except plant protection measures. Three potted cabbage plants were kept in the 80x75x75 cm cage for each treatment. Twenty pairs of freshly emerged DBM moths were released in these cages and the pots were used for imposing the treatments. T1 - *T. chilonis* adults was released twice *i.e.,* 2 and 5 days (each time @ 5 adults plant^{-1}) after release of DBM moths, T2 - *T. chilonis* was released once (@ 5 adults plant^{-1}) 2 days after moth release, followed by a spray of malathion 50 EC (1ml l^{-1}) 7 days later, T3 - two sprays of malathion 50 EC (1ml l^{-1}) was given at 7 and 10 days after release of moths and T4 - untreated control. Five days after release of moths, net cages were removed and pots were left open for further observation. The number of healthy DBM eggs was counted in all the leaves of the potted plants prior to insecticide application to determine the percentage parasitization due to *T. chilonis.* Observations of the surviving larval population on both treated and untreated potted plants were recorded after 1, 3, 5 and 7 days of insecticide applications.

All the experiments were taken up as per CRD design. The treatmental effects was compared with the help of CD value 5 per cent level of significance. Angular and $\log_{10}$ transformations were used to accomplish normality in the data before analysis.

RESULTS AND DISCUSSION

Optimum release dosage of *T. chilonis* on *P. xylostella* eggs

The data summarized in table 1 showed highest parasitization of *P. xylotella* eggs (83.6%) at 100:5 host

parasitoid ratio. The parasitization per cent in higher host parasitoid ratios was lesser than this. The parasitisation per cent at host parasitoid ratios from 100:10 to 100:20 were almost on par with each other but was significantly different from 100:1 ratio showing the least per cent parasitization (40.6%).

The present result get support from the findings of Singh *et al.* (2010), who also noticed highest parasitism at 100:5 host parasitoid ratio while working on *Trichogrammatidae bactrae* Nagaraja in the management of cabbage DBM. Miura (2003) also reported that the percent parasitization of host eggs increased with the increase in the number of parasitoids released. However, the number of parasitized eggs per parasitoid female decreased as the number of released females increased while evaluating the suppressive effect of the egg parasitoid, *Trichogramma chilonis* Ishii on the population density of the diamondback moth. Tabone *et al.* (2006) also reported that parasitism efficiencies of *Trichogramma* strains were higher at the lower egg density than at the higher egg density.

Per cent adult emergence

The percentage adult emergence of *T. chilonis* was maximum (98.6%) at 100:20 host-parasitoid ratio followed by 84 per cent at 100:15 host parasitoid ratio. The results revealed increase in parasitied adult emergence with the increase in the host and released parasite ratio indicating emergence of more than one adult from a single egg. This indicates that with increase in the number of adults, eggs were super parasitized. The variation in parasitism and adult mergence found in the different ratios and being significantly different with increase in host parasitoid ratios may be due to inherent differences in the parasitoid or most likely due to the mutual interference among *T. chilonis* females. Navik and Varshney (2018) reported that *Trichogrammatid* egg parasitoid is widely used in the biological control of lepidopteran pests. Great diversity of *Trichogramma* have been reported worldwide over 230 species parasitizing the different eggs of over 200 insect species belonging to 70 different families. *Trichogramma* and *Trichogrammatoidea* are amenable for laboratory mass production on factitious hosts. Development of a tolerant strain for the different stresses through genetic improvement is considered useful in improving their survival and performance to manage the insect-pests under field conditions.

The mean developmental period of *T. chilonis* in all the host parasitoid ratios were on par with each other ranging from 8.3 to 8.5 days. The mean longevity period of adult *T. chilonis* under different host parasitoid ratios ranged from 3.4 to 3.8 days signifying that all the treatments are on par with each other. The results revealed that developmental period and adult longevity of *T. chilonis* did not differ between treatments.

Table 1: Parasitization potential efficiency of *T. chilonis* at different host and parasitoid ratios on *P. xylostella* eggs

Host parasite ratio	Mean parasitization (%)	Mean developmental period (days)	Mean parasitoid emerged (%)	Mean longevity (days)
100:1	40.6 (39.58)	8.5	30 (5.48)	3.4
100:5	83.6 (66.15)	8.3	60.8 (7.80)	3.4
100:10	71.8 (57.96)	8.3	56.6 (7.52)	3.8
100:15	79.4 (63.05)	8.3	84 (9.17)	3.8
100:20	71.4 (57.68)	8.4	98.6 (9.93)	3.6
SEM (±)	0.34	0.07	0.01	0.12
SE (d)	0.48	0.10	0.01	0.17
$CD_{(p=0.05)}$	1.01	0.21	0.03	0.35

Figures in parenthesis are angular transformed values

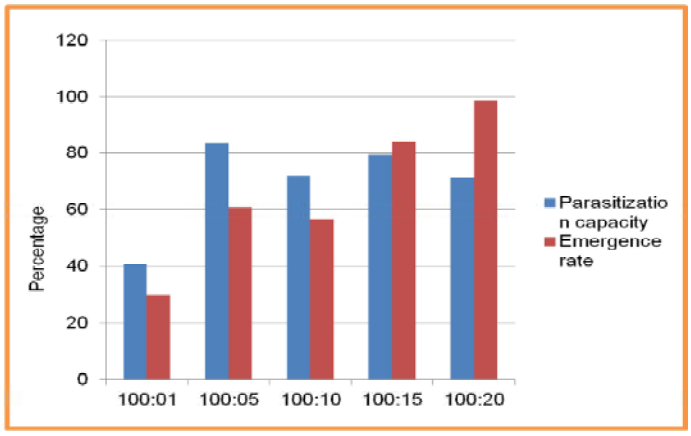

Fig. 1. Graphical representation on parasitization and emergence rate of *T. chilonis* at different host parasitoid ratios on DBM eggs.

Role of *T. chilonis* alone and in combination with malathion in management of *P. xylostella* on cabbage

The data pertaining to management of *P. xylostella by employing T. chilonis* alone and in combination with malathion 50 EC are given in the Table 2. The number of unparasitized eggs of DBM recorded in the treatment with release of *T. chilonis* twice was significantly less (52.8 plant^{-1}) as compared to 166.4, 243.4 and 266.2 in other treatment. This indicates that release of *T. chilonis* twice @ 5 adults plant^{-1} twice (2 and 5 days after DBM moth release) resulted in least unparasitized eggs of DBM. The larval

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00027.8

population of DBM was recorded at 1DAT, 3DAT, 5DAT and 7DAT. The lowest larval population of DBM was recorded (2.8 larvae plant^{-1}) in the treatment with two releases of *T. chilonis* @ 5 adults plant^{-1} (2 and 5 days after DBM moth release) followed by its combination with one spray of malathion 50 EC @1ml l^{-1} (4.8 larvae plant^{-1}) at 1DAT, which were on par.

Similarly, at 3 and 5DAT two releases of *T. chilonis* was found to be the most effective treatment against the pest recording lowest larval population of 5.4 and 6.2 per plant, respectively followed by combined application with malathion 50 EC. The two releases *of T. chilonis* was observed to be superior over other treatments up to 7 days after

Table 2. Efficacy of *T. chilonis* alone and in combination with malathion against *P. xylostella* on cabbage

Treatment	Mean unparasitized eggs of DBM	Mean larval population of DBM plant^{-1}				Pooled mean
		1DAT	3DAT	5DAT	7DAT	
T. chilonis @ 5 adults plant^{-1} twice (2 and 5 days after DBM release)	52.8 (1.72)	2.8 (0.44)	5.4 (0.73)	6.2 (0.79)	6.6 (0.82)	4.6 (0.62)
T. chilonis @ 5 adults plant^{-1} once + one spray of Malathion (1ml l^{-1})	166.4 (2.22)	4.8 (0.67)	7.4 (0.87)	7.6 (0.88)	9 (0.95)	6.55 (0.79)
Two spray of Malathion (1ml l^{-1})	243.4 (2.39)	13.6 (1.13)	15.8 (1.19)	10.2 (1.01)	22.6 (1.35)	15 (1.15)
Untreated control	266.2 (2.42)	37.6 (1.57)	51.8 (1.71)	55.4 (1.74)	59.2 (1.77)	47.45 (1.67)
SE (d)	0.01	0.02	0.01	0.01	0.01	0.01
CD $_{(p=0.05)}$	0.02	0.05	0.03	0.03	0.02	0.02

Figures in parenthesis are $\log_{10}$ transformed values

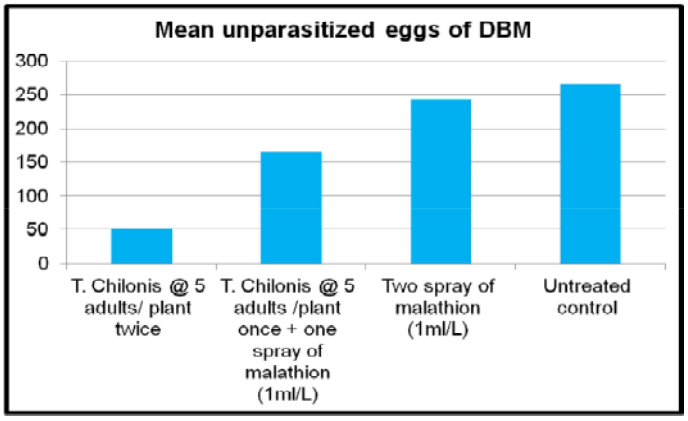

Fig. 2. Graphical representation on unparasitized eggs of DBM

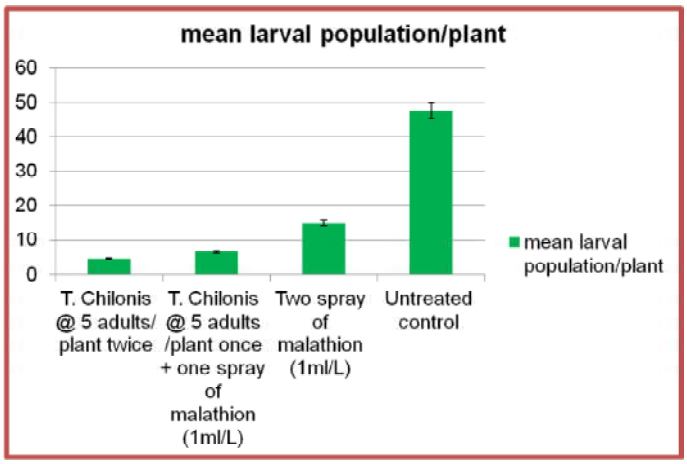

Fig. 3. Graphical representation on larval population per plant

Plate 1. Parasitization efficiency of *T. chilonis* on DBM eggs at different host parasitoid ratio

Plate 2. Releasing of DBM moth inside a cage

treatment, whereas, malathion 50 EC application alone showed its inferiority to other treatments in reducing the larval population in all the observation intervals. All the treatments were significantly superior to untreated control in reducing larval population of DBM. The pooled data also revealed that release of *T. chilonis* @ 5 adults plant^{-1} twice (2 and 5 days after DBM moth release) proved the most effective with the lowest larval population of 4.6 against 47.45 per plant in untreated control. These findings are in agreement with Singh *et al.* (2010) who reported that *T. bactrae* was successfully able to manage *P. xylostella* on cabbage crop.

The results are also supported by Tabone *et al.* (2010)

who evaluated the parasitization potential of various species (and various strains of some species) of *Trichogrammatidae* (Hymenoptera: Chalcidoidea) to control DBM on cauliflower in greenhouse in France. It parasitized 60 per cent or more of the *P. xylostella* eggs. The chi-1 strain of *T. chilonis* and both strains of *T. evanescens* showed the strongest potential for controlling *P. xylostella* on greenhouse cauliflower. Guo *et al*. (1999) also reported *T. chilonis* (Guangdong) as suitable candidates to control DBM in the field. Host location, host acceptance and host suitability are important factors for successful parasitism. The effectiveness of *T. chilonis* releases may be attributed to its greater parasitizing efficiency occurring in the egg stage of DBM and the releases of *T. chilonis* coinciding with egg laying by DBM. The inefficacy of malathion 50 EC may be due to its low persistent nature and its resistance to DBM. Involvement of acetylcholinesterase in malathion-resistance of the diamondback was reported by Maa *et al*. (1997). They showed that monooxygenase and acetylcholinesterase were positively involved in malathion resistance of the MS10 strain of diamondback moth. Maa and Liao (2000) also reported that malathion showed resistance against DBM.

Thus, the suppression of DBM population by application of malathion 50 EC spray alone or in combination with *T. chilonis* showed no efficient effect. The results indicated that application of *T. chilonis* alone can effectively manage diamondback moth population. Therefore, application of *T. chilonis* can be practised as an alternative to using synthetic chemicals to control diamondback moth in cabbage crop.

CONCLUSION

The study concludes that the *Trichogramma chiloris* can be employed as an alternative to pesticides in the managing diamond back moth effectively. The release of parasitoid in host-parasite ratio of 100:5 has shown maximum of 80:3 per cent paraistization of *P. xylostella* eggs.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Entomology, College of Agriculture, CAU, Imphal for providing all the facilities.

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Online Manuscript No. 100441 recived on 06.08.2022
Manuscript accepted for publication on 14.11.2022

Journal of Eco-friendly Agriculture **18**(1): 161-164. 2023
doi: 10.5958/2582-2683.2023.00028.X

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Detection and molecular identification of dominant seed mycoflora associated with fennel cultivars

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ABSTRACT

Fennel (*Foeniculum vulgare* Mill.) is a medicinal plant belonging to the Umbelliferae (Apiaceae) family. The seeds have a fragrant odour and a pleasant aromatic taste. Five different fennel cultivars *viz*., GF 2, GF 11, GF 12, AF 1, and AF 2 were used to detect seed mycoflora. Seven fungal species from five different genera *viz*., *Alternaria alternata, Macrophomina phaseolina, Fusarium oxysporum, Curvularia lunata, Aspergillus niger, A. flavus* and *A. fumigatus* were detected by agar plate method. Among seven different mycoflora, the incidence of *A. alternata* was found more prevalent and it developed more colonies in all five cultivars. Molecular identification of dominant seed mycoflora of fennel cultivars *i.e., A. alternata* was performed *via* PCR amplification and sequencing of the Internal Transcribed Spacer (ITS) region of fungal DNA (rDNA) with universal primer ITS1 and ITS4. The ITS primers produced an amplicon of 530 bp for *A. alternata.* The sequence of the rDNA region (Accession no. ON613536) was aligned and analyzed in the NCBI nucleotide BLAST. It showed 97.94 per cent similarity with already reported *A. alternata* (MN268767.1, MN268766.1, and MH894277.1) sequences.

***Key words*:** Fennel, cultivar, mycoflora, internal transcribed spacer, *Alternaria alternata*

Fennel (*Foeniculum vulgare* Mill.) is a medicinal plant belonging to the Umbelliferae (Apiaceae) family, known and used by humans since antiquity, due to its flavour. The fennel plant is characterized by a hardy, biennial, or perennial herb with yellowish flowers and feathery leaves (Singh, 2017). Fennel may be consumed daily in the raw form as salad and snacks, stewed, boiled, grilled, or baked in several dishes, and even used in the preparation of herbal teas or spirits. Because of its valuable nutritional makeup, including the inclusion of vital fatty acids, a diet rich in fennel could provide beneficial health effects (Dwivedi *et al*., 2008).

India is the world's largest fennel-producing country followed by China, Bulgaria, Iran, Mexico, Syria, and Egypt. In India, fennel is mostly grown in the northern region and the important fennel-producing states are Gujarat, Rajasthan, Madhya Pradesh, Haryana, and Uttar Pradesh. In India, it is grown in an area of 75,000 ha with a production of 1,28,000 MT during 2019-20 (Anonymous, 2020a). Gujarat is the largest fennel-producing state in India followed by Rajasthan. In Gujarat, it is mainly cultivated in Surendranagar, Mehsana, Banaskantha, Patan, Gandhinagar, Aravalli, Kutch, Kheda, and Anand districts. It is cultivated in an area of 52,802 ha with a production of 1,09,026 MT during 2019-20 in Gujarat (Anonymous, 2020b).

The crop harbours many seed-borne fungi as enumerated by Richardson (1990). In India, fungi associated with fennel seeds have been reported from Madhya Pradesh, Rajasthan, Punjab, Uttar Pradesh (Swarup and Mathur, 1972), Bihar (Bilgrami *et al*., 1981), Andhra Pradesh (Regina and Raman, 1988; Singh *et al*., 1991), Maharashtra (Pande and Bangale, 1994) and Haryana (Rani *et al*., 1995). The most predominant fungal genera encountered on fennel seeds were *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., and *Fusarium* spp. (Khanzada *et al*., 2002).

MATERIALS AND METHODS

Detection of dominant seed mycoflora

Five fennel cultivars *viz*., GF 2, GF 11, GF 12, AF 1, and AF 2 were used to detect dominant seed mycoflora. The agar plate method was used to isolate mycoflora from fennel seed samples. The PDA was employed as a basal medium. Twenty ml sterile PDA was put into each Petri plate. After 24 hours of pouring, 10 (9+1) seeds were placed at equidistance on the solidified media. Plates were incubated at 25 ± 2 °C giving a 12/12 alternate cycle of light and darkness of near-ultraviolet (NUV) for 7 days. After incubation, the fungal colonies that appeared on the seed surface were observed under the stereoscopic binocular microscope. Fungal growth was also examined under a compound microscope. Seed mycoflora load in respect of the number of colonies and types of fungi was recorded. All 400 seeds were assessed, as recommended by International Seed Testing Association (ISTA, 1976).

Molecular identification of dominant seed mycoflora

DNA extraction

For DNA extraction, the pathogen was grown on PDA medium at 25 ± 2 °C for 8 days. Mycelia were harvested by filtering through Whatman filter Paper No. 1, washed repeatedly with distilled sterilized water to remove excess salts adhering to it. The DNA extraction was based on the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). Mycelium (1 g) was crushed in liquid nitrogen and transferred into 7.5 ml pre-warmed (65 °C) DNA extraction buffer [1 M TrisHCl (pH 8.0), 5 M NaCl, 0.5 M EDTA (pH 8.0) and 2% CTAB], mixed well and incubated in a water bath at 65 °C with gentle shaking for 45 min. An equal volume of chloroform: isoamyl alcohol (24:1 %v/v) was added and mixed gently to denature proteins and centrifuged at 12,000 rpm for 15 min. at 4 °C. The DNA was precipitated with 0.6 volume of chilled ethanol and 0.1 volume of 3 M sodium acetate (pH 5.2) and centrifuged at 18,514 g for 15 minutes. The pellets were washed twice with chilled 70 % ethanol, dried at room temperature, and re-suspended in 100 ìl sterile TE (10 mM Tris-HCl buffer and 1 mM EDTA-pH 8). Two microliter DNase-free RNase was added to dissolved DNA stock and incubated in a water bath at 37 °C for 45 minutes followed by 65 °C for 10 minutes for enzyme inactivation. The DNA was quantified and qualified using NanoDrop Spectrophotometer and agarose gel electrophoresis. The sample was stored in a deep freezer at – 20 °C temperature for long-term usage.

Sequencing of rDNA-ITS region

The dominant seed mycoflora was identified tentatively on a morphological level and was characterized at the molecular level using rDNA-ITS region sequencing. The ITS 1 and ITS 4-primer pair were used for PCR amplification of the pathogen. The PCR reaction contained 50 ng genomic DNA, 1X PCR buffer (Thermo Scientific), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 ìM of each primer, and 1.0-unit Dream Taq DNA Polymerase (Thermo Scientific) in a 50-ìL reaction volume. The PCR program was: one cycle at 96 °C for 2 min., 35 cycles at 96 °C for 50 sec., 53 °C for 50 sec. and 72 °C for 1 min., and one cycle of 72 °C for 5 min. and then held at 4 °C. The sequencing of the amplified product was carried out in an automated sequencer ABI 3730 genetic analyzer at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka, India.

Genetic data analyses

The sequence identity matrix of dominant mycoflora was generated using Bioedit Sequence Alignment Editor (version 5.0.9) (Hall, 1999). After multiple alignments, phylogenetic analysis was done in MEGA 4.0 (Tamura *et al*., 2007) software using the default parameters of one character-based algorithm. The bootstrapped consensus phylogenetic tree was generated for each of these algorithms with 500 replications.

RESULTS AND DISCUSSION

Detection of dominant seed mycoflora

Results (Table 1) reveals that *Alternaria alternata* was the most dominant mycoflora in all five cultivars (40.97 %) followed by *M. phaseolina* (20.07 %), *A. niger* (18.83 %), *F. oxysporum* (17.82 %), *A. flavus* (17.05 %). In all fennel cultivars, *C. lunata* and *A. fumigatus* had the lowest incidence of 9.39 and 9.85 per cent, respectively.

Molecular identification of dominant seed mycoflora

In the present study, DNA barcoding belonging to dominant fungi *Alternaria alternata* was attempted by targeting the ITS region of fungal DNA (rDNA).

Table 1: Seed mycoflora load on seeds of fennel cultivars by agar plate method

Mycoflora	Seed mycoflora load (%)					Mean (F)
	GF 2	GF 11	GF 12	AF 1	AF 2	
Alternaria alternate	47.20	58.84	32.15	41.12	28.15	40.97
Macrophomina phaseolina	25.22	17.20	18.12	23.39	17.41	20.07
Fusarium oxysporum	21.05	23.18	12.39	18.18	15.31	17.82
Curvularia lunata	10.21	13.21	8.26	9.18	7.19	9.39
Aspergillus niger	18.20	25.13	11.15	19.14	21.46	18.83
Aspergillus flavus	20.22	19.12	10.12	17.12	19.57	17.05
Aspergillus fumigatus	11.16	15.12	9.34	7.24	7.39	9.85
Mean (C)	21.89	23.32	14.50	19.34	16.64	19.14
	C		**F**		**C × F**	
S. Em.±	0.20		0.24		0.54	
C. D. at 5 %	0.57		0.68		1.51	
C.V.%			5.63			

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00028.X

DNA extraction and quantification

The CTAB (Cetyl Trimethyl Ammonium Bromide) DNA extraction methodology was used to isolate DNA, as described in the materials and methods section. The amount of DNA in the sample (2096.38 ng/µl) was measured using NanoDrop Spectrophotometer at A260 nm/A280 nm and A260 nm/A230 nm. For further examination, samples with a high concentration of DNA and no RNA/protein contamination were chosen.

Polymerase Chain Reaction (PCR)

The ITS rDNA region was amplified using universal primer pairs ITS1 and ITS4 for PCR amplification. The primer combination of ITS1 and ITS4 produced an amplicon of 530bp for *A. alternata*. The amplified product was analyzed on 1.8 per cent agarose gel.

Sequencing result of sample

The PCR products were purified and sequenced by Eurofins Genomics India Pvt. Ltd., Bengaluru, India. The results were aligned and examined in the NCBI nucleotide BLAST to determine the similarity of newly sequenced samples to the GenBank databases and they were matched at various global similarity levels. The sequence was submitted to the NCBI GenBank. The ITS rDNA sequence of the sample was confirmed as *A. alternata* region of specific (Accession No. ON613536) covered the internal transcribed spacer-1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer-2, complete sequences, and 28S ribosomal RNA gene, partial sequence. A BLAST search for similarities of *A. alternata* identification showed that the percentage of similarity of the isolate ranged from 96.95 to 98.17 per cent. The pathogen of interest showed 97.94 per cent similarity with already reported *A. alternata* (MN268767.1, MN268766.1, and MH894277.1) sequences.

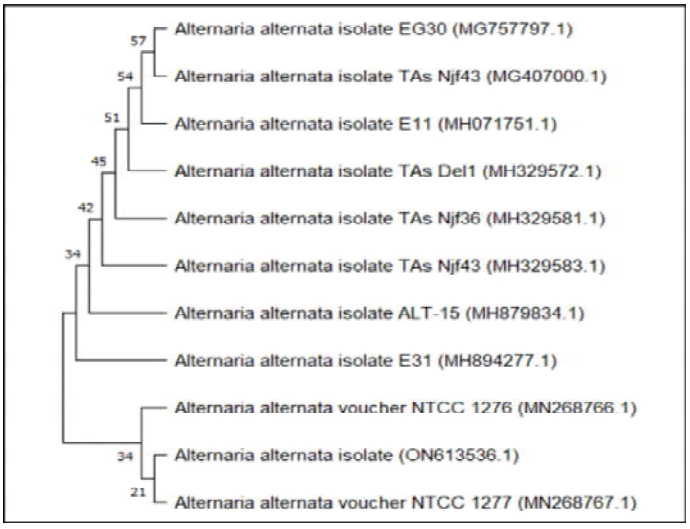

Fig. 1: Phylogenetic tree based on the sequence of rDNA ITS region of *Alternaria alternata*

Phylogenetic analysis

The phylogenetic tree was constructed with nucleotide sequence from the sequenced ITS region of *A. alternata* (Fig. 1) and compared with other similar worldwide fungal isolates available in the NCBI database.

CONCLUSION

The present piece of work concluded that seven different species of fungi *i.e., Alternaria alternata, Macrophomina phaseolina, Fusarium oxysporum, Curvularia lunata, Aspergillus niger, A. flavus, A. fumigatus* were detected from five different fennel cultivars by the agar plate method. *Alternaria alternata* was found more prevalent and it developed more colonies in all five cultivars. Molecular identification through ITS rDNA region of dominant seed mycoflora isolated from fennel cultivars revealed 97.94 per cent similarities with *A. alternata* isolate.

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Online Manuscript No. 100555 recived on 17.8.2022
Manuscript accepted for publication on 19.11.2022

Journal of Eco-friendly Agriculture **18**(1): 165-170. 2023
doi: 10.5958/2582-2683.2023.00029.1

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Efficacy of botanicals and fungicides against *Alternaria* leaf spot of black gram

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ABSTRACT

The study conducted with the aim of evaluating the potential of botanicals, fungicides and its combination in managing *Alternaria* leaf spot disease incited by *Alternaria alternata* in black gram showed maximum inhibition of mycelial growth (77.33%) with metiram + pyraclostrobin and minimum inhibition (27.28%) of mycelial growth with azadirachtin among all the tested treatments. *In vitro* it could successfully manage *Alternaria* leaf spot with 77.37 PDEC and 16.67 PDI. Minimum disease control was expressed by azadirachtin with 34.42 PEDC and 48.33 PDI. The combination of fungicide and the botanicals effectively controled the disease in short duration at low cost. Maximum black gram grain yield (0.90 kg plot^{-1}) was obtained from field applied with combined foliar spray of fungicide and botanical and minimum grain yield was obtained with Foliar spray of azadirachtin (-0.5%) provided minimum (0.56 kg plot^{-1}) grain yield.

Keywords: Black gram, mycelial inhibition, *Alternaria* leaf spots, fungicide, botanical.

Black gram or urd bean *(Vigna mungo),* one of the most economically important legume crops of South-East Asia is adapted to both semi-arid and subtropical areas, is grown in a wide range of agro-climatic conditions in India. It occupies sizable area in India, Bangladesh, Pakistan, Myanmar, Sri Lanka, and West indies. It is grown all over country in *kharif* and summer seasons also, while in south India, it is raised mainly in *rabi* season. The productivity of black gram remains low due to many biotic (mung bean yellow mosaic virus, powdery mildew and *Cercospora* leaf spot) and abiotic stresses (drought, heat and pre-harvest sprouting). It suffers from several diseases, especially *Cercospora* leaf spots (*C. canescens, C. cruenta*), powdery mildew (*Erysiphe polygoni*) and root disease complex (*Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp.). It is affected by several fungal and viral diseases of which *Alternaria* leaf spot is the major reducing annual crop productivity the extent of 20-30 per cent (Trivedi *et al*., 2013) and posing an increasing threat to legume cultivation around the globe including India, causing leaf spot and other diseases on over 380 plant species (Laemmlen, 2001). The pathogen has been reported to attack cauliflower, cabbage, chili, papaya, wheat, rice sorghum etc. causing leaf spot, rots and blight diseases (Rathod, 2012).

Symptoms of this disease appear as small, light brown lesions on leaves and at the tip or the margins, which later turn greyish to dark brown and dull white in centre. With the age of crop and infection time, the lesions enlarge resulting in 'shot hole' symptoms leading to chlorosis, dropping off of leaves and adverse effect on anabolism. In the later stage, the spots become larger in size with distinct concentric rings and dark in colour. Finally, whole plant shows typical blight symptoms. The fungus colonizes the xylem of the host plant resulting in the blockage and breakdown of the xylem and lead to wilt disease symptoms such as, leaf wilting, yellowing and eventually the death of the plant.

In view of the increasing severity of *Alternaria* leaf spot in southern Rajasthan and looking to several factors influencing the efficacy of its management practices, disease development under artificial inoculation in pot culture and to develop a suitable strategy to manage *Alternaria* leaf spot of blackgram, the experiment was conducted.

MATERIALS AND METHODS

In vitro evaluation in amended plain agar media

The efficacy of fungicides, combi products of fungicides and botanicals against *A. alternata* were evaluated *In vitro* by using poisoned food technique. Test combi products, fungicides and botanicals as per the treatments shown in the following table were evaluated on potato dextrose agar (PDA) medium. The flasks containing melted and sterilized PDA was amended with the desired concentration of each test combi products, fungicide and botanical. 20 ml of the amended PDA was poured in 90 mm sterilized petri-plates

and allowed to solidify. From the periphery of 7-days-old actively growing and sporulating culture of *A. alternata*, 5 mm disc was cut and placed in the centre of each experimental petri-plate under totally aseptic conditions.

Treatment details

Treatments	Concentration (%)
Azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC	0.07
Azoxystrobin 23 SC	0.1
Difenoconazole 25 EC	0.06
Metiram 55 per cent + pyraclostrobin 5 per cent WG	0.35
Azadirachtin	0.5
Neem oil	0.5
Control	0

The experiment was conducted in completely randomized design (CRD) with five replications in each treatment and the inoculated plates were incubated at 27±1°C. The colony diameter was measured after 7 days when the control plates were full of fungal growth. Per cent inhibition of mycelia growth was calculated by using formula given by Bliss (1934) as:

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition, C = Colony diameter in control, T = Colony diameter in treatment

***In vivo* evaluation of different fungicides and botanicals**

A field experiment was conducted during *kharif* 2018 at Rajasthan College of Agriculture, Udaipur Instructional Farm in order to estimate the per cent efficacy of disease control and grain yield. The experiment was laid in Randomized Block Design in plot size of 3.6 x 3 m^2. Different test combi products, fungicides and botanicals and their combinations were tested in field. Thirty days old plants were inoculated as foliar spray application having inoculum density of 1×10^{-1} conidia ml^{-1}. The symptoms of *Alternaria* leaf spot started appearing on foliar parts of plants after 36 hours of inoculation of the pathogen. Foliar applications of solutions with desired concentration of fungicides and botanicals individually and in combination was done as per following treatments at initiation of disease and repeated after 15 days intervel. A suitable untreated control was also maintained for comparison.

Treatment details

Name of treatments	Concentrations (%)
Azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC	0.07
Azoxystrobin 23 SC	0.1
Difenoconazole 25 EC	0.06
Metiram 55 per cent + pyraclostrobin 5 per cent WG	0.35
Azadirachtin	0.5
Neen oil	0.5
Fungicide and botanicals most effective *In vitro*	At their respective recommended dose
Control	

Observations of disease severity were recorded on a standard disease rating scale (1-5 score) given by (Sangeetha and Siddaramaiah, 2007).

Disease rating scale given by Sangeetha and Siddaramaiah, 2007.

Scale	Description of the symptoms
1.	Small irregular spots covering <5% leaf area.
2.	Small irregular brown spots with concentric rings covering 5.1-10% leaf area.
3.	Lesions enlarge, irregular brown with concentric rings covering 10.1-25% leaf area.
4.	Lesions coalease to form irregular and appears as a typical blight symptom covering 25.1-50% leaf area.
5.	Lesions coalease to form irregular and appears as a typical blight symptom covering >50% leaf area.

The per cent disease index (PDI) and per cent efficacy of disease control (PEDC) were calculated by using following formula given by Wheeler, 1969:

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all individual disease rating}}{\text{Total No. of plants assessed} \times \text{maximum rating}} \times 100$$

$$\text{PEDC} = \frac{\text{PDI in control - PDI treatment}}{\text{PDI in control}} \times 100$$

(PEDC-per cent efficacy of disease control)

Observations of grain yield (kg/plot)

RESULTS AND DISCUSSION

***In vitro* evaluation in amended plain agar media**

The data presented in Table 1 and Fig. 1 showed that all the fungicides and botanicals caused significant reduction in mycelial growth as compared to control. Irrespective of concentrations, azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC-0.07 per cent, azoxystrobin 23 SC-0.1 per cent, difenoconazole 25 EC- 0.06 per cent, metiram 55 per cent + pyraclostrobin 5 per cent WG- 0.35 per cent, azadirachtin 0.5 per cent and neem oil 0.5

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doi: 10.5958/2582-2683.2023.00029.1

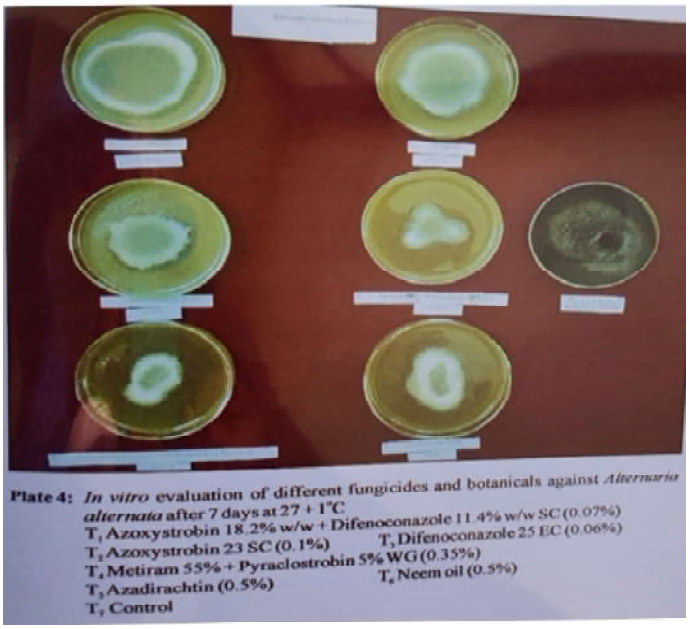
per cent were tested *in vitro*. The results envisage that the combi products metiram 55 per cent + pyraclostrobin 5 per cent WG-0.35 per cent with the colony diameter 20.40 mm was significantly most effective in inhibiting fungal growth followed by azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC- 0.07 per cent, with colony diameter 27.96 mm at given concentrations was again significantly superior in inhibiting the mycelial growth of the test fungus over the individual chemicals and control. Among the individual chemicals, difenoconazole 25 EC-0.06 per cent with colony diameter 36.60 mm was significantly superior in inhibiting the growth of test fungus followed by azoxystrobin 23 SC- 0.1 per cent with colony diameter 38.20 mm. Among botanicals, neem oil 0.5 per cent with 46.05 mm colony diameter was found to inhibit fungal mycelium significantly more than azadirachtin 0.5 per cent with colony diameter 65.45 mm which was found significantly least effective in inhibition of mycelium. All the test chemicals as combi products or individually inhibit the growth of *A.alternata* significantly over botanicals and control. As for the botanicals, the neem oil - 0.5 per cent was significantly more effective within test botanicals with colony diameter 45.90 mm than azadirachtin (-0.5%) with colony diameter of 65.84 mm in inhibiting mycelial growth of *A. alternata.*

Irrespective of concentrations, the metiram 55 per cent + pyraclostrobin 5 per cent WG -0.35 per cent proved to be the most effective in per cent growth inhibitions (77.33) followed by azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC - 0.07 per cent (68.93) in inhibiting the growth of the test fungus over the individual chemicals and control. Among the individual chemicals, difenoconazole 25 EC 0.06 per cent with the per cent growth inhibition 59.33 was significantly superior in inhibiting the growth of test fungus followed by azoxystrobin 23 SC 0.1 per cent with the per cent growth inhibition 57.56 and neem oil - 0.5 per cent was significantly more effective within test botanicals with the per cent growth inhibition of 48.83 than azadirachtin -0.5 per cent with the per cent growth inhibition of 27.28 in inhibiting the growth of *A.alternata* (Table 1 Plate 4 and Fig. 1).

Table 1: *In vitro* efficacy of different fungicides and botanicals against *A. alternata* after 7 days after inoculation at 27±1°C

Treatments	Colony diameter (mm)*	Per cent growth inhibition**
Azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC (0.07)	27.96	68.93 (56.13)
Azoxystrobin 23 SC (0.1)	38.20	57.56 (49.35)
Difenoconazole 25 EC (0.06)	36.60	59.33 (50.38)
Metiram 55 per cent + pyraclostrobin 5 per cent WG (0.35)	20.40	77.33 (61.57)
Azadirachtin (0.5)	65.45	27.28 (31.48)
Neem oil (0.5)	46.05	48.83 (44.33)
Control	90.00	0.00
SEm±	0.651	0.724
CD at 5%	1.887	2.097
CV %	3.14	3.34

* Mean of five replications
Figures in parentheses are arcsine per cent angular transformed values.



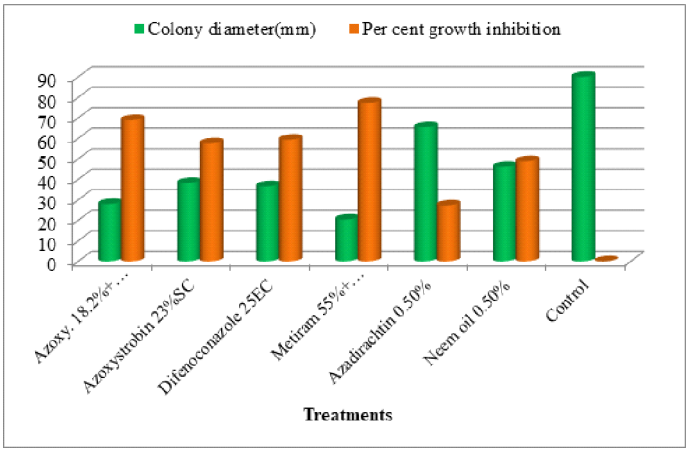


Fig. 1: *In vitro* efficacy of different fungicides and botanicals against *A. alternata* after 7 days after inoculation at 27±1°C

Evaluation of different fungicides and botanicals in field conditions

All the foliar applications of fungicides and botanicals were significantly superior to individual foliar application treatments. The data revealed that significantly maximum PDI (73.67) of *Alternaria* leaf spot infection was observed in the untreated control. The result revealed that the treatment having combination of fungicide and botanical, which are most effective *In vitro* with PDI 16.67, was most effective against *A. Alternate* which was significantly higher as compared to individual applications of test combi products, individual fungicides, botanicals and untreated control. Among combi-products tested, Metiram 55 per cent + pyraclostrobin 5 per cent WG -0.35 per cent with the PDI 18.33 followed by azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC - 0.07 per cent, with PDI 19.67 at given concentrations were significantly superior in managing the disease over the individual chemicals and botanicals. Among the individual chemicals, difenoconazole 25 EC0.06 per cent with PDI 25.33 was significantly superior in managing the disease followed by azoxystrobin 23 SC 0.1 per cent with PDI 29.00. Among botanicals, neem oil - 0.5 per cent with PDI 38.33 exhibited significantly superior over azadirachtin -0.5 per cent with PDI 48.33. All the test chemicals as combi products or individually and botanicals managed the disease significantly better over the control. As for botanicals neem oil - 0.5 per cent was significantly more effective within test botanicals with PDI 38.33 than azadirachtin-0.5 per cent with PDI 48.33 in managing *Alternaria* leaf spot of black gram (Table 2, Plate 5 and Fig. 2).

Maximum per cent efficacy of diseases control (PEDC) of *Alternaria* leaf spot of black gram was observed in combined foliar spray of fungicide and botanical which were most effective *In vitro* against *A. Alternate* with PEDC 77.37. This treatment exhibited significant superiority over individual applications of test combi products, individual fungicides and botanicals and untreated control. Among combi products tested metiram 55 per cent + pyraclostrobin 5 per cent WG -0.35 per cent with the PEDC 75.11 followed by azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC - 0.07 per cent with PEDC 73.30 at given concentrations were significantly superior in managing the disease over the individual chemicals and botanicals. Among the individual chemicals, difenoconazole 25 EC 0.06 per cent with PEDC 65.60 was significantly superior in managing the disease followed by azoxystrobin 23 SC 0.1 per cent with PEDC 60.62. All the test chemicals as combi products or individually managed the disease significantly over botanicals and control. As for botanicals, neem oil - 0.5 per cent was significantly more effective within test botanicals with PEDC 47.92 than azadirachtin -0.5 per cent with PEDC 34.42 in management of *Alternaria* leaf spot of Black gram (Table 2 and Fig. 2).

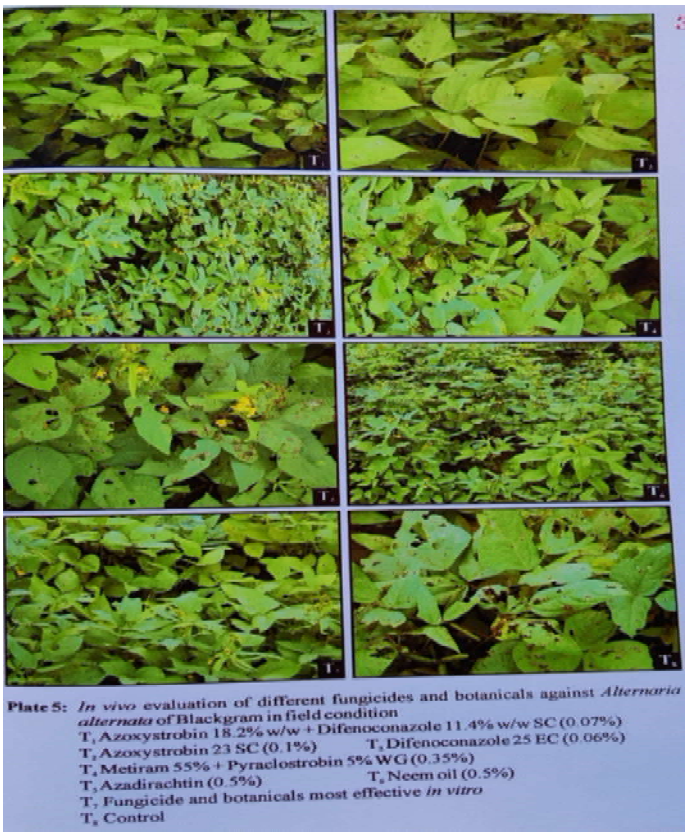
Table 2: *In vivo* evaluation of different fungicides and botanicals against *Alternaria* leaf spot of black gram

Treatments	Per cent disease Index (PDI*)	Per cent efficacy of disease control (PEDC**)	Grain yield (kg plot^{-1})
Azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC (0.07)	19.67 (26.33)	73.30 (47.46)	0.87
Azoxystrobin 23 SC (0.1)	29.00 (32.56)	60.62 (51.15)	0.77
Difenoconazole 25 EC (0.06)	25.33 (30.22)	65.60 (54.09)	0.81
Metiram 55 per cent + pyraclostrobin 5 per cent WG (0.35)	18.33 (25.35)	75.11 (60.08)	0.88
Azadirachtin (0.5)	48.33 (44.04)	34.42 (35.90)	0.56
Neem oil (0.5)	38.33 (38.23)	47.92 (43.80)	0.67
Fungicide and botanicals most effective *In vitro*	16.67 (24.09)	77.37 (61.60)	0.90
Control	73.67 (59.13)	0.00	0.28
SEm±	1.410	1.889	0.037
CD at 5%	4.228	5.664	0.112
CV %	7.26	6.03	9.03

* Mean of three replications

Figures in parentheses are arcsineÖper cent angular transformed values.

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00029.1



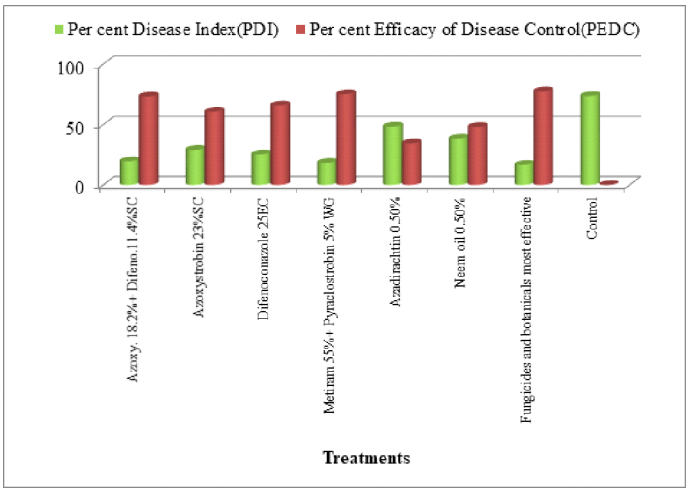


Fig. 2: *In vivo* evaluation of different fungicides and botanicals against *Alternaria* leaf spot of black gram

Maximum grain yield of black gram was obtained from the field treated with combined foliar application of fungicide and botanical and were the most effective *in vitro* against *A. alternata* with 0.90 kg plot^{-1} grain yield. This treatment exhibited significant superiority over individual

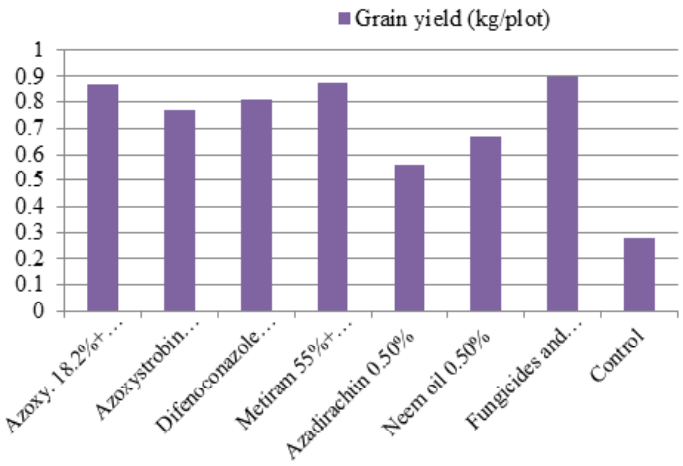


Fig. 3: Grain yield (kg plot^{-1})

applications of test combi products, individual fungicides and botanicals and untreated control. All combi products tested, metiram 55 per cent + pyraclostrobin 5 per cent WG - 0.35 per cent and azoxystrobin 18.2 per cent w/w + difenoconazole 11.4 per cent w/w SC - 0.07 per cent with grain yield 0.88, 0.87 kg plot^{-1}, respectively, at given concentrations were significantly superior in grain yield over the individual chemicals and botanicals. Among the individual chemicals, difenoconazole 25 EC 0.06 per cent with grains yield 0.81 kg plot^{-1} was significantly superior in grain yield followed by azoxystrobin 23 SC 0.1 per cent with grain yield 0.77 kg plot^{-1}. All the test chemicals as combi products or individually were found in produce grains significantly more over botanicals and control. As for botanicals, neem oil - 0.5 per cent was significantly more effective within test botanicals with grain yield 0.67 kg plot^{-1} than azadirachtin -0.5 per cent with grain yield 0.56 kg plot^{-1} in black gram field (Table 2 and Fig. 3).

Surviliene and Dambrauskiene (2006) reported efficacy of azoxystrobin 250SC against various *Alternaria* species *viz*, *A. alternata, A.brassicae, A.dauci* on potato dextrose agar under *in vitro* conditions and reported 87.0 per cent to 96.0 per cent inhibition of colonies of different *Alternaria* species by azoxystrobin at 7 days after incubation. Gawade *et al.* (2016) studied management of *A. alternata* incidence due to captan + mancozeb (@ 0.2% each) treatment was 85.96 per cent over control. Similar results on the efficacy of plant extracts against *Alternaria* spp. have been reported by Rao (2006) reported a combi product iprodione + carbendazim @ 0.2 per cent as effective fungicide for the management of *Alternaria* blight of sunflower. Extracts of other plants like neem, onion, tulsi and datura were also found effective against diseases caused by *A. alternata*.

CONCLUSION

The study concludes that among the two combi products, two fungicides and two botanicals evaluated initially *In vitro* and in field on black gram crop, the metiram (55%) + pyraclostrobin 05 per cent WG (0.35%) recorded the maximum inhibition of mycelial growth of *A. alternata* (77.33%). The treatment combinations of fungicide and botanicals found most effective *in vitro* could also successfully manage *Alternaria* leaf spot in the field also with 77.37 PDEC and 16.67 PDI. and minimum disease control was expressed by Azadirachtin 0.5 per cent with 34.42 PEDC and 48.33 PDI. The treatment effectively controlled the disease in short duration at a low cost. Foliar spray of fungicide and botanical which were most effective *In vitro* against *A. alternata* produced maximum black gram grain yield (0.90 kg plot^{-1}).

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Online Manuscript No. 97104 recived on 10.3.2022
Manuscript accepted for publication on 16.7.2022

Journal of Eco-friendly Agriculture **18**(1): 171-175. 2023
doi: 10.5958/2582-2683.2023.00030.8

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Morphological characterization and *in vitro* efficacy of bioagents against *Alternaria alternata* blight of pigeonpea

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ABSTRACT

The *Alternaria* blight of pigeonpea is an important foliar disease posing serious threat to pigeonpea cultivation due to change in the climatic condition, cultivation practices and variation in pathogenic character. Hence, the effective management of the pathogen needs its characterization and identification of effective bio-agent for the farming community. Among 20 isolates characterized for morphology, conidia of different isolates were separated by one or two vertical septa and 2-8 hortizontal septa and the maximum horizontal septa of 3-8 was exhibited by the isolates PLS-4 and PLS-10, respectively and the maximum size of the conidia was revealed by PLS18 and PLS19 with 226.52-394.60 mm × 75.40-105.40 mm and 233.04-374.48 mm × 71.40-101.44 mm, respectively. The isolate PLS 18 showed maximum beak length of 44.40-190.70 mm. Among the bioagents tested under dual culture, *T. hamatum* was found most effective and statistically significant over other bio-control agents in inhibiting the mycelial growth (82.78%) of *Alternaria alternata* followed by *T. viride* (78.61%).

Key words: *Alternaria alternata*, bio-agents, *in-vitro*, morphology, pigeonpea

Pigeonpea [*Cajanus cajan* (L.) Millsp.] (2n=2x=22) belongs to the genus *Cajanus*, subtribe *Cajaninae*, tribe *Phaseoleae* and family *Fabaceae*. The word *Cajanus* is derived from a Malay word '*Katschang*' or '*Katjang*' meaning pod or bean. Several authors considered Eastern Africa to be the center of its origin occuring in the wild form (Zeven and Hukovsky, 1975). However, De (1974) and Vernon (1976) while reviewing the pigeonpea origin concluded that India is the primary and Africa is the secondary center of origin. Pigeonpea is predominantly grown in *Kharif* season both as a sole and intercrop under a wide range of agro-ecological situations. The deep root system of pigeonpea helps in extracting the nutrients and moisture from deeper soil layers and making it suitable for rainfed conditions and enables in breaking of the plough pans. Besides this, it fixes atmospheric nitrogen and thereby increases soil fertility (Reddy, 1990; Smita *et al*., 2015) and improves the soil structure and hence called 'Biological Plough'.

The global pigeonpea production scenario accounts for an area of 6.99 m. ha. with the production of 5.96 m. t. and productivity of 852 kg ha^{-1} (Anon., 2018). In India, the total area coverage, production and productivity accounts for 4.78 m. ha., 3.59 m. t. and 751 kg ha^{-1}, respectively. The state-wise trend showed that Maharashtra ranks first both in area and production (29.19% and 29.68%) followed by Karnataka (19.23% and 15.96%). Even though the pigeonpea accounts for about 90 per cent of the world area and production, there are constraints in productivity over the years. This might be due to abiotic and biotic factors. Among more than 200 pathogens known affecting the crop, 83 are fungi (Nene *et al*., 1989). The leaf blight caused by *Alternaria alternata* is economically important and widespread causing heavy losses in parts of Northern Karnataka.

Nevertheless, an effective disease management programme would be designed by understanding the biology of the pathogen which needs an accurate identification of pathogen and its characterization. The eco-friendly management using effective bio-control agents would be of immense help in managing the disease.

MATERIAL AND METHODS

Collection of samples, isolation and artificial inoculation of pathogen

Blight infected pigeonpea samples were collected from Raichur, Kalaburgi, Bidar, Koppal, Ballari, Yadgir, Vijayapur and Bagalkot districts of Karnataka during the field survey carried out in *Kharif* 2015 to *Kharif* 2017. The pathogen *Alternaria* spp., was isolated from the blighted leaves. Initially, the infected leaves were cut into small pieces of 1 to 2 mm size and surface sterilized with mercuric chloride (0.1%) for one minute and subsequently washed three times with sterile distilled water and transferring the bits to petri dishes

containing sterilized potato dextrose agar (PDA) medium. Inoculated plates were incubated at 25 ± 2 ! for colony growth. The cultures were purified by hyphal tip isolation and cultures were morphologically identified as *Alternaria alternata* based on standard mycological parameters and rDNA sequencing and maintained on PDA slant at 4 ! for further study. The cultural and morphological characters *viz*., colony colour, shape, texture, growth pattern and type of mycelial growth was observed. Dimensions of the conidia were measured using binocular microscope fitted with the Gryphax image analyser and capture software and the mean measurements were calculated at 40X magnification.

Pigeonpea variety, TS-3R grown under glasshouse was used for pathogenicity studies. Ten days old seedlings were inoculated with 250 ml of *Alternaria* spore suspension as described by Sharma *et al.* (2013). Pure culture of the *A. alternata* was inoculated into 100 ml of potato dextrose broth (PDB) and incubated at 25 ± 2 ! for 7 days. The conidia were harvested and suspended in sterile distilled water. Seedlings were inoculated by spraying the spore suspension (5×10^6 conidia ml^{-1}). The inoculated seedlings were covered by polythene bags and incubated at 28 ± 1 ! with alternate day and night photoperiod and un-inoculated plants served as control. After 24 hrs, polythene covers were removed and regularly monitored for disease development.

Diversity in morphological characters

The diversity in morphological characters of 20 isolates of *A. alternata* collected from surveyed fields of Northern Karnataka were studied. The morphological characters of the fungus, such as mycelial width, size of the conidia and the length of beak were measured under high power objective (40X) using binocular microscope fitted with the gryphax image analyser and capture software.

Efficacy of bio-agents against *A. alternata* under *in vitro*

Bioagents *viz*., *Trichoderma viride, T. harzianum, T. virens, T. hamatum, T. piluliferum* and *P. fluorescens* (Ep-5) obtained from biocontrol laboratory, Department of Plant Pathology, UAS, Raichurw were evaluated for their efficacy under *in vitro* using dual culture technique against *Alternaria alternata*. The mechanism of inhibition of pathogen by the bioagents was studied to identify the efficient bio-agent against the pathogen.

Dual culture test

Bio-agents were evaluated for their efficacy through dual culture technique, the fungal bio-agents and the test pathogen were inoculated in the periphery in an opposite direction of the single petri dish containing solidified PDA medium. Whereas, the bacterial bio-agents were streaked one day prior to the pathogen inoculation. Four replications were maintained and one control by maintaining pathogen and bio-agent alone and incubated for nine days. The colony diameter of both the bio-agents and the pathogen was measured in both the directions and the average was recorded. The per cent growth inhibition of the test pathogen was calculated by using the formula given below by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition

C = Growth in control

T = Growth in treatment

Invert plate technique

Bio-agents were also evaluated for their efficacy through inverted plate technique to know volatiles role in inhibiting the pathogen growth, the fungal bio-agents and the test pathogen were inoculated in the two different lower lids of petri plate containing solidified PDA medium. After inoculation, both the inoculated petri plates were placed one over the other and wrapped with the parafilm. Three replications were maintained and one control by maintaining pathogen and bio-agent alone and incubated for nine days. The colony diameter of both the bio-agents and the pathogen was measured in both directions and the average was recorded. Per cent growth inhibition of the test pathogen was calculated by using the formula given by Vincent (1947).

RESULTS AND DISCUSSION

Characterization of pathogen causing blight of pigeonpea

The fungus *Alternaria* produced greyish brown to black coloured colony with cottony to velvety texture and raised to flat mycelial growth. The fungus produced abundant branched septate brownish mycelia. The conidia were solitary or in short chains produced on simple conidiophores. The conidial mean measurement was in the range of 171.54-312.02 × 64.65-141.22 µm with beak and beakless, ovate to ellipsoidal and some were elongated and branched in chains. The conidia had 2 to 8 horizontal septa and 1 to 5 vertical septa. Based on the morphological characters, the fungus was identified as *Alternaria alternata*. In total, 20 isolates (two from Raichur, seven from Kalaburgi, one from Bidar, two from Koppal, two each from Ballari, Koppal, Yadgir, Vijayapur and Bagalkot districts) were isolated and maintained as pure culture. The pathogenicity of the isolated fungus was confirmed on the cultivar TS-3R

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doi: 10.5958/2582-2683.2023.00030.8

by artificially spraying 250 ml spore suspension. The inoculated plants produced typical symptoms at seven days of incubation as light brown concentric circular ring spots and later they turned to dark brown coloured and coalesced to cause blighting of leaves.

Diversity in morphological characters

Twenty isolates of *A. alternata* obtained from different geographical regions were studied for their diversity in morphological characters such as mycelial width, length and width of conidia, beak length and number of horizontal and vertical septa. The conidia of different isolates are separated by one to two vertical and 2–8 horizontal septa. The dimensions of conidia are presented in Table 1 and Plate 1. The isolates PLS 4 and PLS 10 showed maximum horizontal septa of 3–8, whereas minimum horizontal septa were observed in isolate PLS 11 (2-3). The vertical septa of most of the isolates ranged from 0 to 1. The isolates PLS 18 and PLS 19 showed maximum size of 226.52-394.60 mm X 75.40-105.40 mm and 233.04-374.48 mm X 71.40-101.44 mm, respectively. The minimum size of the conidia was observed in isolate PLS 11 (152.88-209.28 mm X 75.68-90.20 mm). The length of the beak of isolates ranged from 0.00-26.10 mm to 44.40-190.70 mm. The isolate PLS 18 showed maximum beak length of 44.40-190.70 mm. The minimum beak length was observed in isolate PLS 6 (5.30-23.80 mm). The mycelial width was larger in the isolate PLS 12 (26.10-124.50 mm) and minimum in PLS 1 (19.00-33.33 mm). The findings of the present investigation is supported by the findings of Raja and Reddy (2007), who collected the samples of leaf spot and fruit rot caused by *A. alternata* from brinjal growing areas and it was found that the size of conidia varied from 35.2-43.5 µm and 12.4-13.9 µm wide, with average beak length of 9.6 -12.4 µm. Horizontal and vertical septations of conidia varied form 1.8 and 0.3, respectively and conidia were produced in chain.

Efficacy of fungal and bacterial bio agents against *A. alternata* by dual culture technique

Six bio-agents were tested against the inhibition of mycelia growth of *A. alternata* through dual culture (Table 2 and plate 2). Among the bioagents tested, *T. hamatum* was found more effective and statistically significant over other bio-control agents in inhibiting the mycelial growth (82.78%) of *A. alternata* followed by *T. viride* (78.61%) and they were significantly superior over other treatments. However, the least mycelial inhibition was recorded in *P. fluorescens* (44.68%).

Efficacy of fungal and bacterial bio agents against *A. alternata* by invert plate technique

Five bio-agents were tested against the inhibition of

Table 1. Morphological diversity of 20 isolates of *A. alternata* on pigeonpea

Isolates	Size of conidia (µm)				Septa in conidia		Length of the beak (µm)		Mycelial width (µm)	
	Length (40X)		Width (40X)		HS	VS	Range	Mean	Range	Mean
	Range	Mean	Range	Mean						
PLS 1	180.08-248.52	197.22	60.12-104.48	75.86	2-5	0	4.92-22.6	14.73	19.00-33.33	24.09
PLS 2	158.60-356.80	265.49	71.40-130.60	102.38	2-4	0	6.92-20.85	15.23	23.80-38.63	29.82
PLS 3	153.60-249.80	186.18	76.80-101.32	90.57	3-4	0-2	12.40-25.20	17.59	15.80-38.30	31.01
PLS 4	168.40-335.20	254.46	66.00-110.72	83.65	3-8	0	36.70-50.90	43.00	34.30-52.70	47.82
PLS 5	164.92-284.52	232.03	50.12-74.60	66.33	3-7	0-1	8.30-62.00	36.94	29.10-56.00	37.58
PLS 6	161.20-260.12	195.78	64.12-102.32	77.56	3-4	0-2	5.30-23.80	14.40	19.00-47.10	28.94
PLS 7	154.92-195.40	175.26	65.20-115.52	91.75	2-4	0	0.00-29.10	16.18	23.80-43.60	34.90
PLS 8	160.92-280.48	231.30	54.00-74.00	66.18	5-6	0-1	34.80-49.70	44.61	34.30-41.10	38.54
PLS 9	180.48-260.00	201.26	60.12-106.32	74.33	2-5	0-1	40.60-62.30	52.58	21.10-48.60	32.23
PLS 10	165.32-290.20	222.61	65.00-120.72	82.98	3-8	0	30.22-49.90	41.86	29.50-50.30	42.16
PLS 11	152.88-209.28	171.54	75.68-90.20	83.78	2-3	0-1	0.00-26.10	16.32	15.80-38.30	30.96
PLS 12	145.40-366.80	237.73	86.00-140.00	115.70	3-5	0-1	29.10-45.50	37.02	26.10-124.50	55.84
PLS 13	190.32-280.52	231.34	47.40-73.00	64.65	2-5	0	38.80-49.00	44.39	29.10-40.30	32.09
PLS 14	161.04-276.24	230.41	52.48-73.00	65.57	4-5	0-1	6.92-21.60	15.27	18.60-40.25	28.23
PLS 15	186.00-281.60	239.91	62.00-95.80	82.05	3-6	0-1	41.60-119.90	70.06	23.80-66.60	49.06
PLS 16	167.40-365.12	276.69	73.92-143.80	98.62	3-5	0	44.80-50.70	47.78	23.80-41.60	37.54
PLS 17	170.92-371.80	302.79	86.40-211.40	141.22	3-5	0-1	55.40-150.80	98.94	29.10-47.40	35.52
PLS 18	226.52-394.60	302.59	75.40-105.40	90.73	3-6	0-1	44.40-190.70	84.10	29.88-41.66	34.95
PLS 19	233.04-374.48	312.02	71.40-101.44	87.16	4-6	0-1	42.56-125.30	66.86	21.10-33.33	28.23
PLS 20	142.00-248.12	189.62	95.80-127.12	109.26	3-5	0-1	29.10-40.30	35.26	23.40-51.70	35.32
	S. Em±	22.94		8.14				9.34		5.54
	CD at 1%	64.60		22.96				26.32		15.63

HS- Horizontal septa VS-Vertical septa

doi: 10.5958/2582-2683.2023.00030.8

mycelia growth of *A. alternata* through invert plate (Table 2 and plate 3). Among the bioagents tested, *T. piluliferum* was found more effective and statistically significant over other bio-control agents in inhibiting the mycelial growth (72.22%) of *A. alternata* followed by *T. hamatum* (58.51%) and *T. viride* and they were statistically on par with each other and significant over other treatments and control. However, the least mycelial inhibition was recorded in *T. harzianum* (9.63%).

Chemicals are spectacular, impressive, quick and convincing even to an uneducated farmer, but there is an intensified worldwide concern about environmental pollution due to escalated use of hazardous pesticides including fungicides also. A multitude of microbes has been implicated to be bio-control agents of the plant pathogen, sometimes with excellent documentation. Hence studies were conducted to find out an effective biocontrol agent against *A. alternata* and to develop a biocontrol technique as a feasible component in the present day integrated disease

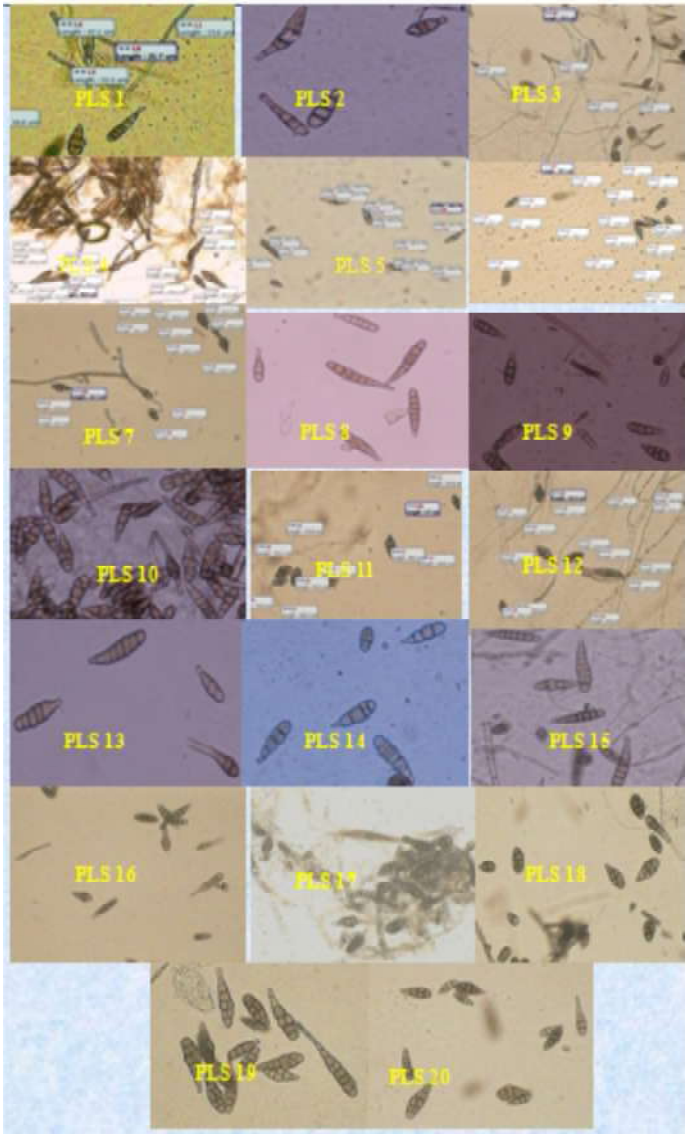

Plate 1. Morphological variability of 20 isolates of *A. alternata* collected from of Northern Karnataka

Plate 3. Efficacy of bioagents in inhibiting the *A. alternata* by dual culture assay

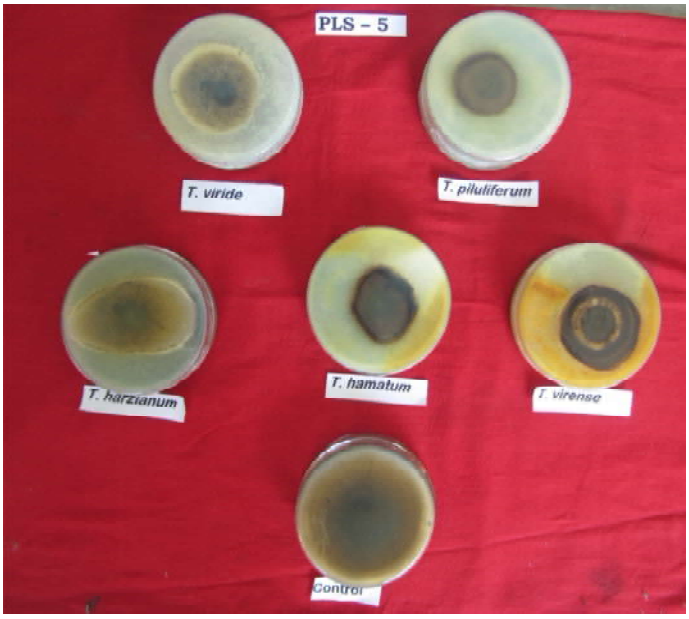

Plate 3. Efficacy of bioagents in inhibiting the *A. alternata* by invert plant technique

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00030.8

Table 2. Efficacy of bio-agents against the growth of *Alternaria alternata* under dual culture and invert plant technique

Bio-agents	Per cent inhibition*	
	Under dual culture	Invert plate technique
Trichoderma viride	78.61 (62.45)	57.40 (41.01)
T. piluliferum	76.67 (61.12)	72.22 (47.39)
T. harzianum	72.78 (58.55)	9.63 (15.49)
T. virense	76.67 (61.12)	51.11 (38.25)
T. hamatum	82.78 (65.48)	58.51 (41.49)
Pseudomonas fluorescens (Ep-5)	49.45 (44.68)	- -
S. Em±	0.26	0.79
CD at 1%	1.10	2.41

*Mean of four replications.

management (IDM) strategy. In the present investigations, bacterial antagonist and potential fungal bioagents were tested and among the tested bioagents, *T. hamatum* (82.78%) was found more effective as compared to other biocontrol agents in mycelial inhibition of *A. alternata* under dual culture and *T. piluliferum* (72.22%) under invert plate technique.

These findings are similar to that of research conducted by other researchers (Thaware *et al*., 2010, Rani *et al*., 2018 and Kayim *et al*., 2018). Their findings revealed that *T. viride* and *T. harzianum* as potential bioagents in inhibiting the mycelial growth of *A. alternata*. The present findings are also supported by the work carried out by Vasudha *et al*., 2018. The results of finding revealed that, the *T. hamatum* and *T. viride* were effective in inhibiting the mycelial growth of *A. alternata* causing leaf spot of pomegranate. The mechanisms for bio-control of plant pathogens by *Trichoderma* are antibiosis, competition and mycoparasitism. In the present finding, *T. hamatum T. viride* and *T. piluluferum* suppressed the growth of *A. alternata* and that might be due to mutual intermingling of antagonistic isolate with the test pathogen and also the volatiles produced by the antagonists might have inhibited the mycelial growth of test pathogen. The findings showed effectiveness of antagonistic activity of *T. hamatum*. *T. viride* and *T. piluliferum* against *Alternaria alternata*.

CONCLUSION

The experiment concluded that the bioagent, *T. hamatum* was most effective and statistically significantly over others in inhibiting mycelial growth (83.78%) of *Alternaria alternate* followed by *T. virdae* (78.61%).

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Online Manuscript No. 97153 recived on 13.3.2022

Manuscript accepted for publication on 5.11.2022

Journal of Eco-friendly Agriculture **18**(1): 176-178. 2023
doi: 10.5958/2582-2683.2023.00031.X

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Efficacy of essential oils on cercospora leaf spot caused by *Cercospora canescens* in black gram

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ABSTRACT

Field studies, aimed at to evaluate the effectiveness of bio-agents and essential oils such as neem oil, clove oil, garlic oil, zinger oil and NSKE against *Cercospora canescens* in *In-vivo* conditions, showed that *Trichoderma viride* + clove oil @ 2.5 per cent caused significant reduction is *C. canescens* disease intensity leading to considerable increase in growth attributes *viz.,* no. of leaves, no. of branches, plant height, no. of flowers, no. of pods followed by T. virdae + garlic oil (2.5%).

Keywords: Black gram, bio-agents, *Cercospora canescens*, essential oils, *Trichoderma viride.*

Black gram (*Vigna mungo* L.) is also known as urd bean, mash and black maple an annual, semi-erect spreading herb belonging to the family fabaceae and is grown during the *Kharif* season in all tropical and sub-tropical regions across the globe. It is high in nutrient value *viz.,* protein (24%), fat (1.4%), carbohydrate (59.6%), calcium (154mg), phosphorous (385mg), iron (9.1mg), beta carotene (38mg), thiamine (0.4mg), riboflavin (0.37mg) and niacin (2mg) per 100g seeds (Aggarwal *et al.,* 2019). It is also used as a high nutritive fodder for milch cattle. Being a deep-rooted crop, it binds with the soil particles enhancing the soil structure and texture by preventing soil erosion. The crop used as a green manure crop also fixes atmospheric N @ 42 kg ha^{-1} $year^{-1}$ in the soil and improves the soil fertility.

The sustainable cultivation is continuously challenged by several biotic factors resulting in losses in quality and quantity of the produce. Among biotic factors, the anthracnose (*Colletotrichum lindemuthianum*), bacterial leaf blight (*Xanthomonas phaseoli*), cercospora leaf spot (*Cercospora canescens*) and corynespora leaf spot (*Corynespora phaseoli*), powdery mildew (*Erysiphe polygoni*), root rot and web blight (*Rhizoctonia solani*) and stem canker (*Macrophomina phaseolina*) play a key role in crop growth. Among these, the cercospora leaf spot has been reported to incur economic losses of about 50-70 per cent (Lal *et al.,* 2001); (Chand *et al.,* 2012); (Aggarwal *et al.,* 2019) and (Ryley *et al.,* 2010). It is reported that the disease in severe cases can damage upto 50 per cent plants (Singh *et al.,* 2010). As most of the management practices followed are chemical based that leave harmful impact on environment and the mankind the study to evaluate the eco-friendly combinations, mentioned in table 1, against cercospora leaf spot in black gram was undertaken.

Table 1. Details of the treatment combinations

	Details of the treatments	Mode of application
T_0	Control	-
T_1	*Trichoderma viride* + neem oil @2.5%	Seed treatment
T_2	*Trichoderma viride* + NSKE @2.5%	Seed treatment
T_3	*Trichoderma viride* + clove oil @2.5%	Seed treatment
T_4	*Trichoderma viride* + garlic oil @2.5%	Seed treatment
T_5	*Trichoderma viride* + ginger oil @2.5%	Seed treatment

MATERIALS AND METHODS

The experiment was conducted at the central research farm, Division of Plant Pathology, Naini Agricultural Institute, Prayagraj during *Rabi* season in 2021.

Plants showing typical symptoms in the field of the standing crop of black gram were identified, selected and brought to the laboratory for further studies.

Examination of fungal colony characteristics was done through microscopic examinations. A small portion of the diseased leaf portion was detached from the leaf surface and placed on a sterile glass slide, stained with lactophenol and cotton blue for a better view. Then, the glass slide was carefully placed under the microscope and the morphological characteristics of the fungal pathogen were observed. The conidiophores are in fascicles, with a length of 20-200×3-6.5µm, pale brown and mostly found on the lower leaf surface and usually elongate, producing successive conidia. Conidia are hyaline with 50-150×3-5.5µm in length, 5-15 septate, smooth, acicular to obclavate, straight or curved, apex acute, basal cell truncate with a distinct scar (Fig. 2).

The disease symptoms were initially observed on lower leaves as a small, brown and hydrolysed circular spots surrounded by a yellow hallow that later enlarged and formed patches of ¼ - ⅛ inch in diameter with irregular, light brown and slightly sunken with a rough, scabby centre. These spots coalesce and lead to death (Fig. 3).

The infected seeds, leaves and pod husk were hypothesized as possible sources of primary inoculum for the disease recurrence. The pathogen is soil-borne on diseased plant debris and survives as a parasite only on the tissues to about 3m through the soil in a season, apparently along with roots. The fungus survives on infected plant stubble for 2.5years in vertosols and upto 3 years in alfisols.

Disease intensity of the plants was observed randomly and scored for disease severity by following the 0-9 scale as in table.2 (Kapadiya and Dhruj 1999) and per cent disease intensity was calculated by using Wheeler 1969 formula.

Table 2: Rating scale: (Kapadiya and Dhruj, 1999)

Grade	Leaf area covered	Reaction
0-1	Free from disease.	Immune
2	Traces to pinhead size spots on leaves	Highly resistant
3	Spots slightly larger than pinheads	Resistant
4	Spots occupying 2-5% of leaf area.	Moderately resistant
5	Spots occupying 5-10% of leaf area.	Moderately susceptible
6	Spots occupying 10-25% of leaf area	Susceptible
7	Spots occupying 25-50% of leaf area	Susceptible
8	Spots occupying 50-75% of the leaf area	Highly susceptible
9	Spots occupying more than 75% of the leaf area.	Highly susceptible

$$\text{Disease intensity (\%)} = \frac{Sum\ of\ numerical\ disease\ ratings}{No.\ of\ plants\ observed \times Maximum\ disease\ rating} \times 100$$

Trichoderma viride was isolated from the soil by serial dilution technique and culture proliferation was achieved by the single spore technique. Thereafter, 4g of mycelium was mixed with talc powder. The powder formulation so obtained was used for seed treatment. The treatment combination of *Trichoderma viride* and different essential oils, 2.5g of talc-based *Trichoderma viride* powder and a drop of detergent were added to 97.5ml of water in a conical flask to form 100 ml of solution. The desired seeds were soaked in the solution for 20-30 mins and sown.

RESULTS AND DISCUSSION

Effect on disease intensity (%)

The statistical analysis of the data (table 3) revealed minimum disease intensity in treatment T_3 (*T. viride* + clove oil @2.5% (24.25%), followed by T_4 (*T. viride* + garlic oil @2.5% (31.56%), T_1 (*T. viride* + neem oil @2.5% (34.54%), T_5 and T_2. All the treatments were significant over control and among themselves, similar findings are also reported with the combination of clove oil (Raja Gopal Reddy *et al.,* 2009; Sharma *et al.,* 2021 and Amrita *et al.,* 2020).

Table 3: Effect of treatments on disease intensity (%)

Details of the treatments		Disease intensity (%)		
		30 DAS	45 DAS	60 DAS
T_0	Control	19.87	34.25	59.13
T_1	*Trichoderma viride* + neem oil @2.5%	12.42	25.78	34.54
T_2	*Trichoderma viride* + NSKE @2.5%	19.82	28.27	37.77
T_3	*Trichoderma viride* + clove oil @2.5%	10.84	16.66	24.25
T_4	*Trichoderma viride* + garlic oil @2.5%	12.15	25.57	31.56
T_5	*Trichoderma viride* + ginger oil @2.5%	14.35	28.08	35.52
	S. Ed (±)	0.45	0.48	0.45
	C. D@ (5%)	0.07	1.04	0.96

Effect on plant height (cm)

The date presented in the table.4 represents that treatment T_3 – *T. viride* + clove oil @2.5 per cent was most effective in achieving maximum plant height (31.10cm), followed by T_4 – *T. viride* + garlic oil @2.5 per cent (30.15cm), T_1, T_5 and T_2 compared to control. All the treatments were significant over control but, among the treatments (T_3, T_4) (T_4, T_1), (T_1, T_5 and T_2) and T_0 were non-significant to each other. Similar findings with seedling treatment with *T. viride* + clove oil is reported by (Amrita *et al.,* 2020). They observed that the combination is most effective in increasing plant growth parameter.

Effect on no. of branches

The statistical results presented in the table.5 reveals that among the treatments T_3 – *T. viride* + clove oil @2.5 per cent (27.41) could significantly increase the no. of branches followed by T_4 – *T. viride* + garlic oil @2.5 per cent (25.87%), T_1 – *T. viride* + neem oil @2.5 per cent (23.42), T_5 – *T. viride* +

Table 4: Effect of treatments on plant height (cm)

Details of the treatments		Plant height (cm)		
		15 DAS	30 DAS	45 DAS
T_0	Control	4.90	15.49	27.69
T_1	*Trichoderma viride* + neem oil @2.5%	7.97	19.17	29.52
T_2	*Trichoderma viride* + NSKE @2.5%	5.70	17.29	28.79
T_3	*Trichoderma viride* + clove oil @2.5%	12.13	22.08	31.10
T_4	*Trichoderma viride* + garlic oil @2.5%	8.10	19.81	30.51
T_5	*Trichoderma viride* + ginger oil @2.5%	7.27	18.99	29.32
	S. Ed (±)	0.62	0.21	0.46
	C.D @ (5%)	1.38	0.45	1.02

Table 5: Effects of treatments on no. of branches

Details of the treatments		No. of branches		
		30 DAS	45 DAS	60 DAS
T_0	Control	2	16	23
T_1	*Trichoderma viride* + neem oil @2.5%	5	16	23
T_2	*Trichoderma viridei* + *NSKE @2.5%*	4	11	20
T_3	*Trichoderma viride* + clove oil @2.5%	8	21	27
T_4	*Trichoderma viride* + garlic oil @2.5%	6	18	26
T_5	*Trichoderma viride* + ginger oil @2.5%	5	15	21
	S. Ed (±)	0.75	0.48	0.19
	C.D @ (5%)	1.68	1.06	0.41

Table 6: Effect of treatments on no. of pods

Details of the treatments		No. of pods
T_0	Control	14
T_1	*Trichoderma viride* + neem oil @2.5%	17
T_2	*Trichoderma viride* + NSKE @2.5%	15
T_3	*Trichoderma viride* + clove oil @2.5%	17
T_4	*Trichoderma viride* + garlic oil @2.5%	17
T_5	*Trichoderma viride* + ginger oil @2.5%	16
	S. Ed (±)	0.79
	C.D @ (5%)	1.75

ginger oil @2.5 per cent (21.38) and T_2 – *T. viride* + NSKE @2.5 per cent (20.35) compared to control. All the treatments were significant over untreated control and each other. Similar findings were reported by (Sileshi *et al.,* 2014).

Effect on no. of pods

The statistically analysed results depicted in the table.6 shows that among all the treatments, the treatments T_1 (*T. virdae* + neem oil @2.5 percent, most effective with 17 pods in each were most effective followed by T_3 – *T. viride* + clove oil @2.5% and T_4 (*T. viride* + garlic oil @2.5%) with T_1–T_5 (*T. viride* + ginger oil @2.5%) and T_2 – (*T. viride* + NSKE @2.5%) with 15 pods, respectively as compared to control. All the treatments were found significant over untreated control and each other, Similar findings were reported by (Sileshi *et al.,* 2014), who reported that treatment combination with clove oil is highly effective in increasing the number of pods in black gram.

The effectiveness of *T. viride* in managing *C. canescens* in black gram may be attributed to its mycoparasitic nature. The clove oil also reported as.

CONCLUSION

The study concludes that the essential oils and bio-control agents have great affinity in inhibiting the pathogen growth and simultaneously enhancing the crop growth. The combination of *Trichoderma viride* and clove oil was found the best and most effective treatment in managing the disease intensity than the remaining treatment combinations.

ACKNOWLEDGEMENTS

The authors are thankful to the head of the department, Department of plant pathology, Naini Agricultural Institute, Prayagraj, U.P.

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Online Manuscript No. 98259 recived on 24.5.2022
Manuscript accepted for publication on 15.11.2022

Journal of Eco-friendly Agriculture **18**(1): 179-184. 2023
doi: 10.5958/2582-2683.2023.00032.1

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Effect of solid culture media and cultural or morphological variability among different isolates of *Macrophomina phaseolina* stem and root rot of sesame (*Sesamum indicum*)

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ABSTRACT

Five isolates of *Macrophomina phaseolina* (Tassi) Goid. namely, AUMP-1, AUMP-2, AUMP-3, AUMP-4 and AUMP-5 were isolated and identified morphologically. Variability among these isolates of *M. phaseolina* was determined using growth parameters and sclerotial body formation criteria. Based on the virulence study on the susceptible host (VRI-1) it was identified that AUMP-1 was the most virulent with (53.60% of disease severity) and AUMP- 2 was the least. Optimum growth of the *M. phaseolina* was observed at 30 ^{o}C temperature and observed maximum mycelial growth (90.00 mm) under *in vitro* conditions. Cultural and morphological studies revealed that the mycelium of *M. phaseolina* initially appeared as dirty white then turned to fluffy white to black with minute dark black sclerotia on the PDA medium. The hyphal branches were at dense and feathery angles with constriction of hyphal branches at their point of origin with the closed septum. The microsclerotia were dark black in colour and varied in size from 70.27 to 99.00 µm. The highest radial growth and excellent sclerotial formation were obtained on PDA media (90.00 mm) and proved best for growth followed by Richard's Agar media (83.63 mm) for growth of *M. phaseolina*.

***Key words*:** Sesame, isolates, cultural, morphological, variability, *M. phaseolina*

Sesame (*Sesamum indicum* L.) is a diploid (2n = 26) dicotyledonous, belongs to the family *Pedaliaceae*, and is the most ancient oil crop in the world (Weiss *et al*., 1983). It is native to Africa region but now it is grown in tropical regions around the world and cultivated for its edible seeds. Sesame is commonly known as "Till" and also known as the "Queen of Oil seeds". It contributes 25 per cent of global production. Among oil seed crops cultivated in India, sesame occupies the fourth place. In India, sesame was cultivated during *kharif* 2020-21 in 15.26 lakh ha area with a production of 7.49 lakh tonne and having productivity of 491 kg/ha (Anonymous, 2020). Among all sesame-growing states Uttar Pradesh, Madhya Pradesh, Rajasthan and Gujarat account for nearly 85 per cent of the area and 82 per cent of the production of the country (Anonymous, 2020).

Charcoal rot caused by *Macrophomina phaseolina* affects severely all stages of crop growth. *Macrophomina phaseolina* is a diverse, omnipresent soil-borne pathogen which causes dry root rot on a number of economically important crops *i.e*., vegetables, pulses, oil seeds and fruit crops (Viana and De Souza, 2002). Seedling mortality due to seed-borne infection aggravates the disease problem by reducing the plant stand per unit area, resulting in low yield and 5-100 per cent yield loss have been reported by Vyas and Woodside (1984). Murugesan and Moravcsik (1978) observed 1.8 kg yield loss per hectare at every one per cent increase in disease intensity. This disease reports up to 50 per cent incidence resulting in heavy yield losses (Chattopadhyay and Kalpana Sastry, 1998). In severe conditions, losses have been reported up to 67 per cent (Kumar *et al*., 2006).

The mycelium of the fungus is dirty white, growing with branched septate hyphae bearing a number of sclerotia. It is highly variable with isolates differing in microsclerotia size and presence and absence of pycnidia. The pycnidia are initially immersed in host tissue, then erumpent at maturity. They are dark to greyish becoming black with age, globose or flattened membranous to sub-carbonaceous with an inconspicuous or definite, truncate ostiole. The pycnidia bear simple rod-shaped conidiophores. Conidia are single-celled hyaline and oval. Microsclerotia are jet brown to black in colour and appear smooth externally, variable in shape: round, oblong, elliptical, curved, and irregular or even forked and varying in size, 25 x 22 - 152 x 32 um, these are produced abundantly in the infected host tissues and are of uniform in texture, more or less loosely packed. Microsclerotia are formed from aggregates of hyphal cells joined by a melanin material with 50-200 individual cells composing individual microsclerotia (Huda-Shakirah *et al*., 2019).

The pathogen survives in harsh conditions by the formation of sclerotia and dormant mycelium in crop

residues and soil. Isolates collected from different parts of Rajasthan are characterized by their colony parameters *viz*., colony colour, colony appearances, sclerotial size and shape and their spore formation. *Macrophomina phaseolina* is a seed and soil-borne pathogen, so its management by cultural and morphological characteristics was observed and classified into different categories.

Keeping in view the importance of the disease, the present study was undertaken to identify the most effective fungicides in controlling stem and root rot disease of sesame.

MATERIALS AND METHODS

To study the cultural and morphological variability

The root rot diseased samples were collected from different locations *i.e*., Pipar city of Jodhpur district, Samdari of Barmer district, Sojat of Pali district and Kheenvsar of Nagaur district. All five isolates (Table 1) were grown on Potato Dextrose Agar (PDA) medium and cultural characteristics like colony diameter, colour and growth pattern were studied. The observation of colony colour and texture was recorded on the 7th day of incubation. The required quantity of the above-mentioned solid medium was prepared and sterilized at 121 °C at 1.1 kg/cm^2 pressure for 20 minutes. Sterilization of Petri dishes was done at 160-180 °C for 2 hours in a hot air oven. In each Petri dish, 25 ml of medium was poured. Each treatment was replicated three times. Each Petri dish was inoculated with a mycelial bit of 5 mm diameter maintained on plain agar. The inoculated Petri dishes were incubated at 27±1 °C temperature and observations of mycelial growth were recorded accordingly.

Table 1. Isolates of *Macrophomina phaseolina* from different sesame growing areas

Sl. No.	Pathogen Isolated from	District Name	Isolate code
1.	Jodhpur	Pipar city	AUMP-01
2.	Barmer	Samdari	AUMP-02
3.	Pali	Sumerpur	AUMP-03
4.	Nagaur	Kheenvsar	AUMP-04
5.	Jaisalmer	Jaisalmer	AUMP-05

Preparation of Slides

A small amount of pure culture was taken using a sterile needle and transferred onto a clean sterile slide. The culture of each isolate was taken from four positions of the culture plate. A total of three culture plates of each isolate were used for the morphological studies after 10 days of incubation at 27±1 °C. The culture was stained with 0.1% lactophenol cotton blue and observations on different morphological characteristics were taken and recorded for each of the isolates.

Morphological variability

The slides of various isolates were prepared from 10-day old culture for studying morphological characteristics *viz*., size of hyphal length, hyphal width, mycelial colour, size of sclerotia, number of sclerotia, colour of sclerotia and shape of sclerotia were recorded after 10 days of incubation by using Mycaps image analyser.

Cultural variability

The colonies of isolates were characterized for growth rate at 72 h after incubation. Seven days old cultures were used to record the texture, the colour of the colony and the presence or absence of aerial mycelium.

Pathogenic variability

For assessing the pathogenic variability of *M. phaseolina* isolates sick pot soil method (Salunkhe and Deshpande, 2014) was followed. The potting mixture was prepared thoroughly by mixing clay loam soil, sand and farm yard manure at 1:1:1 ratio. The inoculum of each isolate of *M. phaseolina* collected from different locations was separately mixed at 5 per cent level (w/w) with the sterilized soil filled in 30 cm earthen pots ten days before sowing. Surface sterilized (using 0.1% mercuric chloride solution for 30 seconds followed by two washings in sterile water) sesame seeds were sown at 30 seeds per pot. Three replications were maintained in a completely randomized block design and the sesame cultivar RT-351 was used in this study. The pots were maintained in a glass house with regular, judicious and uniform watering. The root rot incidence was recorded at 35 days after sowing (DAS) and the per cent disease incidence was calculated and isolates were grouped as Least virulent (< 20%), Virulent (21-50%), and Highly Virulent (> 50%) according to per cent disease incidence as suggested by Gupta *et al*. (2012).

Mass multiplication of the pathogen

The inoculum of the test pathogen, *M. phaseolina* maintained on agar slants was further multiplied on sorghum grains. Sorghum seeds of 100 g were washed thoroughly in tap water and soaked overnight in 250 ml conical flask with the addition of 20 ml of 4 per cent dextrose. The flasks were then autoclaved for 20 min at 15 lbs. After cooling the flask at room temperature they were shaken well to separate the sterilized grains and were inoculated with 2-3 discs of the 4-day-old culture of *M. phaseolina* and incubated at 27 ± 1 °C for 7 days in BOD incubator. After 7 days, the inoculum was mixed with sterilized soil in pots at 5 per cent level (w/w).

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00032.1

Effect of solid culture media and temperature on the growth of different isolates of *Macrophomina phaseolina*.

Growth of different isolates on the solid culture media

With a view to finding out the best medium for the growth and sclerotial formation of fungus, six media in solid states were compared. The compositions of various media used are as under.

1. Czapek's Agar Medium (CzAM)

Sodium nitrate ($NaNO_3$)	: 2.0 g
Dipotassium hydrogen orthophosphate (K_2HPO_4)	: 1.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	: 0.50 g
Potassium chloride (KCl)	: 0.50 g
Ferrous sulphate ($FeSO_4.7H_2O$)	: 0.01 g
Sucrose ($C_6H_{22}O_{11}$)	: 3.00 g
Agar agar	: 20.00 g
Distilled water	: 1000.00 ml

2. Oat Meal Agar (OMA)

Oats	: 100.00 g
Agar agar	: 20.00 g
Distilled water	: 1000.00 ml

3. Peptone Sucrose Agar Medium (PSAM)

Sodium glutamate	: 1.0 g
Peptone	: 10.0 g
Sucrose	: 10.0 g
Agar agar	: 17.0 g
Water	: 1000 ml

4. Potato Dextrose Agar (PDA)

Peeled potato	: 200.00 g
Dextrose ($C_6H_{12}O_6$)	: 20.00 g
Agar agar	: 20.00 g
Distilled water	: 1000.00 ml

5. Richard's Agar Medium (RAM)

Potassium nitrate (KNO_3)	: 10.00 g
Potassium dihydrogen orthophosphate (KH_2PO_4)	: 5.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	: 2.5 g
Ferric chloride ($FeCl_3.6H_2O$)	: 0.02 g
Sucrose ($C_6H_{22}O_{11}$)	: 50.00 g
Agar agar	: 20.00 g
Distilled water	: 1000.00 ml

6. Sabouraud Agar Medium (SAM)

Peptone	: 10.0 g
Dextrose	: 40.0 g
Agar agar	: 15.0 g
Water	: 1000 ml

The cultural study was conducted on PDA in Petri plates (90 mm). Sterilized media (20 ml) was poured into each sterilized Petri plate. The plates were inoculated with the fungal disc of 5 mm size cut from the periphery of 10-day old pure culture of *M. phaseolina* and incubated at 27±1 °C. Three replications were maintained for each culture medium. Colony diameter was measured diagonally after 3 days of inoculation. Mycelial growth and number of sclerotia and size were recorded after 7th day of incubation. The observations on Mycelial growth (mm) and Sclerotial formation were recorded (Table 2).

Table 2. Criteria used to record the sclerotial formation of *Macrophomina phaseolina*

Sl. No.	Particulars	Sclerotial production/ microscopic field	Sign indication
1	No sclerotial formation	Nil	-
2	Poor sclerotial formation	1-20	+
3	Moderate sclerotial formation	21-30	+ +
4	Good sclerotial formation	31-50	+ + +
5	Excellent sclerotial formation	>50	+ + + +

Growth of different isolates at different temperature

Six different temperature levels *viz*., 15, 20, 25, 30, 35 and 40 °C were used for these studies. Observations on average colony diameter and sclerotial formation were recorded.

RESULTS AND DISCUSSION

Variability of different isolates of *Macrophomina phaseolina*

Morphological and cultural characteristics

The cultural and morphological characteristics such as colour and appearance of the colony, mycelial growth, branching pattern, size and shape of their sclerotia of different isolates of *M. phaseolina* were recorded by growing them on PDA medium. The results presented in Plates 1, 2, and 3 revealed that there were notified variations in colony growth rates of five isolates of fungus (measured at 7 days after inoculation) were observed and the colony growth rate of these five isolates followed the sequence of descending order as AUMP-1 (99.56 mm) > AUMP-5 (99.36 mm) > AUMP-3 (95.50 mm) > AUMP-2 (84.23 mm) > AUMP-4 (79.12 mm).

Based on the colony appearance AUMP-1, AUMP-3 and AUMP-5 are characterized as fast-growing isolates whereas AUMP-2 is a moderate-growing isolate and AUMP-4 is a slow-growing isolate. The isolates also showed a different type of colony colour and appearance. AUMP-1 appeared as blackish in colour with excellent sclerotial formation whereas AUMP-5 emerged as whitish and whitish creamy in colour, respectively without any sclerotial formation. AUMP-4 showed round-shaped sclerotia whereas AUMP-1 observed oblong-shaped sclerotia. The AUMP-2 isolates developed an oval shaped whereas AUMP-3 and AUMP-5 showed irregular shaped sclerotia under compound microscopy. The AUMP-1 and AUMP-4 showed a dense type of branching pattern whereas AUMP-2, AUMP-3 and AUMP-5 showed a feathery type of branching pattern. However, they had significant variation in the size of sclerotia ranged from 70.27-99.00 µm. The maximum size of sclerotia was reported with AUMP-4 (93.27 µm) followed by AUMP-3 (90.55 µm), AUMP-5 (81.97 µm). The lowest size of sclerotia on culture media was observed in isolates AUMP-2 (80.65 µm) and AUMP-1 (70.27 µm).

Similar findings were also supported by Riaz *et al*. (2007) in sunflower. Shekhar *et al*. (2012) observed a direct correlation between sclerotial production and virulence of isolates in the case of charcoal rot on maize and collected seven isolates which were characterized by their cultural and pathogenic characteristics. Kaur *et al*. (2013) worked on 61 isolates of *M. phaseolina* in pigeon pea and differentiated them on the basis of their morphological and cultural characteristics. Iqbal and Mukhtar (2014) reported 65 isolates and analyzed that the radial growth of the pathogen ranged from 32.00 to 87.17 mm and significant variation was detected among 65 isolates on their radial growth, sclerotial size, weight as well as pathogenicity test. Satpathi and Gohel (2018) and Wagan *et al*. (2018) also worked on the same pathogen and collected different isolates, and categorized these on their morphological and cultural features in terms of colony colour, radial growth, shape and size of sclerotia.

Pathogenic variability

Pathogenic variability among different isolates of stem and root rot of sesame pathogen was tested under field conditions. All isolates were tested on a popular cultivar (RT-351). All five tested had a significant variation in the appearance of disease symptoms which started to emerge at 26 days after sowing in isolate AUMP-1 to 50 days after sowing in isolate AUMP-2. The appearance of disease symptoms (days after sowing) of these five isolates on sesame crop followed the sequence of ascending order as AUMP-2 (26) < AUMP-1 (27) < AUMP-3 (29) < AUMP-5 (30) < AUMP-4 (35). All five isolates also recorded significant variation in disease incidence (18.51-56.00%) under field conditions except isolates AUMP-1 and AUMP-4. The isolate AUMP-1 was found highly pathogenic among all isolates and recorded the highest disease incidence (56.00%) followed by AUMP-4 isolate (49.23%). The lowest disease incidence was observed under AUMP-2 (18.51%) followed by AUMP-1 isolate (20.00%).

Similar findings were also reported by Sharmishha *et al*. (2004) on cluster beans. Reyes-Franco *et al*. (2006) carried out pathogenic and genetic characterizations between 96 isolates of *M. phaseolina* from different hosts in Mexico and other countries and found that 15 isolates had few pathotypes, 9 isolates had zero and 59 isolates showed 61% pathotypes. Famiyeh and Mlsna (2017) and Gade *et al*. (2018) collected 40 isolates from soybean fields that were infected with root rot and found that the Rb-29 was highly virulent among all isolates. Kumar *et al*. (2019) collected 16 isolates and worked on pathogenic variability.

Effect of solid culture media and temperature on the growth of different isolates of *Macrophomina phaseolina*

Growth of *Macrophomina phaseolina* isolates on the solid culture media

Six different solid media were tested for their suitability for the mycelial growth and sclerotial production of the test pathogen. The data are presented in Table 3 and Plate 2.

Among the six solid media tested, the highest mycelial growth was recorded on PDA (90.00 mm) followed by Richard's agar (83.63 mm). The next best media in order of merit were Oat Meal Agar (73.75 mm), Czapek's agar (62.50 mm) and Peptone sucrose agar (59.25 mm) medium and the lowest growth (52.50 mm) was recorded in Sabouraud agar medium.

The excellent sclerotial formation of *M. phaseolina* was observed on PDA and Richard's agar medium. The good sclerotial formation was recorded on Oat Meal Agar, while

Table 3. Mycelial growth and sclerotial formation of *Macrophomina phaseolina* on different solid media *in vitro*

Sl. No.	Medium	Average colony diameter (mm)	Sclerotial formation*
1	Czapek's Agar	62.50	++
2	Oat Meal Agar	73.75	+++
3	Peptone Sucrose Agar	59.25	+
4	Potato Dextrose Agar	90.00	++++
5	Richard's Agar	83.63	++++
6	Sabouraud's Agar	52.50	++

* + Poor, + + Moderate, + + + Good, + + + + Excellent

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00032.1

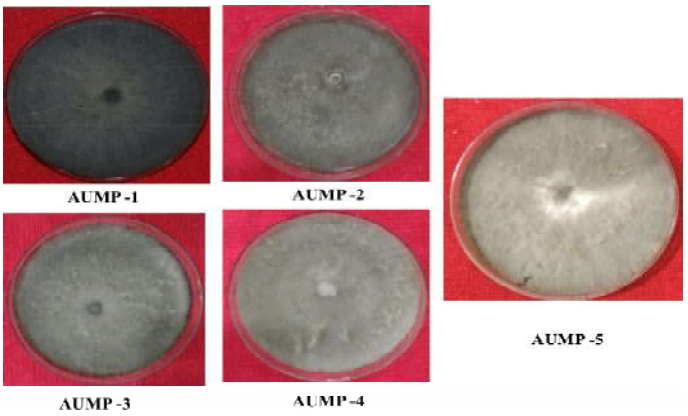

Plate 1: Pure culture of *Macrophomina phaseolina* by different isolates

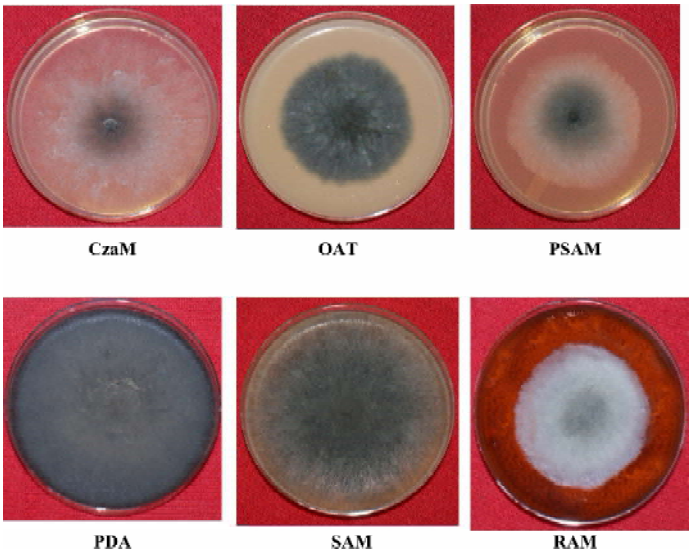

Plate 2: Growth of different isolates on the solid culture media

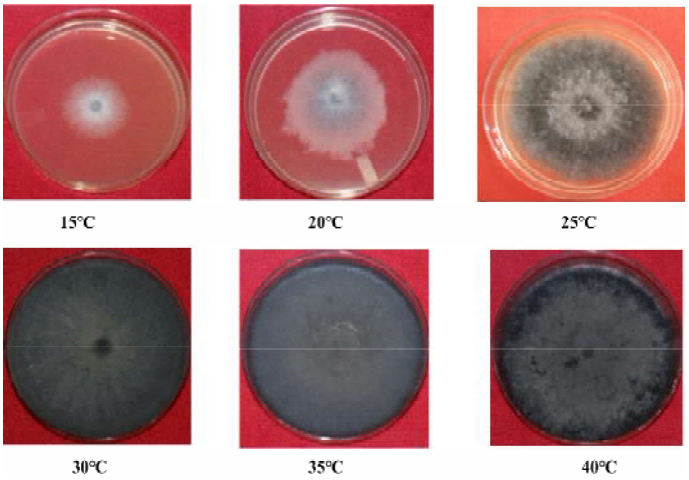

Plate 3: Mycelial growth of different isolates at temperature *in vitro*

moderate sclerotial formation was observed on Czapek's Agar and Sabouraud Agar. Peptone Sucrose Agar revealed very poor sclerotial formation.

The results of the present investigation are similar to the results obtained by earlier workers. Grover and Sakhuja (1981) reported that the pycnidial stage was never produced in an artificial medium. Sobti and Sharma (1992) reported that the fungus well grown on PDA medium with similar cultural characteristics of mycelium and sclerotial formation were found after 3rd and 7th day of incubation, respectively confirming our results. Suriachandraselvan *et al.* (2003) also reported the best radial growth and excellent sclerotial formation of *M. phaseolina* on PDA and Richard's agar medium

Growth of *Macrophomina phaseolina* isolates at different temperatures

Six different temperature levels *viz*., 15, 20, 25, 30, 35 and 40 °C were used for the study. To determine the optimum temperature for the mycelial growth of different five isolates of *M. phaseolina,* were grown at different six temperature levels on PDA medium. The results presented (Table 4 & Plate 3) indicates that the mycelial growth of each isolate of *M. phaseolina* grew best at 30 °C temperature (88.42 to 90.00 mm) followed by 35 °C temperature (87.20 to 88.90 mm) on 8th day of incubation. There was very poor mycelial growth (23.50 to 32.65 mm) at 15 °C temperature in all the test isolates on the 8th day of incubation.

Jha and Sharma (2005) reported that the optimum temperature for *Rhizoctonia bataticola* was 30-35 °C, both for mycelial growth and sclerotial formation. At 40 °C temperature, very poor mycelial growth and no sclerotium

Table 4. Mycelial growth of *Macrophomina phaseolina* isolates at different temperatures *in vitro*

Sl. No	Different isolates	Temperature/ Mycelial growth (mm)*					
		15 °C	20 °C	25 °C	30 °C	35 °C	40 °C
1	AUMP-1	26.75	56.25	72.30	90.00	85.75	80.12
		(31.13)	(48.57)	(58.22)	(71.73)	(67.78)	(63.47)
2	AUMP-2	23.50	56.65	70.65	88.50	86.50	82.50
		(28.98)	(48.80)	(57.17)	(70.14)	(68.41)	(65.24)
3	AUMP-3	30.75	51.25	76.95	89.25	88.90	79.30
		(33.66)	(45.69)	(61.28)	(70.83)	(70.51)	(62.91)
4	AUMP-4	31.25	53.50	77.50	90.00	87.20	81.65
		(33.96)	(46.98)	(61.66)	(71.84)	(69.01)	(64.61)
5	AUMP-5	32.65	55.75	74.90	88.42	85.00	80.70
		(34.82)	(48.28)	(59.91)	(70.08)	(67.19)	(63.91)
	S.Em.±	0.467	0.416	0.225	0.263	0.270	0.337
	C.D. at 5%	1.491	1.328	0.719	0.839	0.863	1.075
	C.V. (%)	2.489	1.512	0.655	0.642	0.683	0.911

*Average of three replications, Figures in parentheses are angular transformed values

were observed in their study. Sandhu and Singh (1999) and Sharma *et al*. (2004) also reported higher temperature ranges of 25-30 °C favoured the growth of *M. phaseolina*. Naik *et al*. (2010) also reported that 30 °C and 35 °C were congenial for optimum mycelial growth (90 mm and 89.50 mm, respectively) of *M. phaseolina.*

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Online Manuscript No. 98232 recived on 22.5.2022
Manuscript accepted for publication on 19.11.2022

Journal of Eco-friendly Agriculture **18**(1): 185-187. 2023
doi: 10.5958/2582-2683.2023.00033.3

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Molecular characterization of *Fusarium* sp. from maize seeds

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ABSTRACT

The investigation was undertaken at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, India during 2021-22. Genetic identification was done to identify the fungal pathogen and Internal Transcribed Spacer (ITS) rDNA was employed to identify the fungal pathogen at the species level. The molecular identification of the dominant seed mycoflora of maize *i.e. Fusarium oxysporum* was performed via, PCR amplification and sequencing of the ITS region of fungal DNA (rDNA) with universal primer pairs ITS1 and ITS4. The ITS primers produced an amplicon of 547 bp which was purified and sequenced. The sequences of rDNA region (Accession No. ON637065.1) were aligned and analyzed in the NCBI nucleotide BLAST. It showed 99.16% similarity with the already reported *F. oxysporum* (MN272279.1, MK751702.1, MF063025.1, KF534741.1, and JN585764.1) sequences.

Key words: Internal transcribed spacer, polymerase chain reaction, NCBI, seed mycoflora, maize, cultivar

Maize (*Zea mays* L.) is the world's most widely grown cereal crop having been domesticated in Central America. Fungi affect the quality of grain through the increase in fatty acid, reduction in germination, mustiness, and finally spoilage of grain. Fungal development in grains is influenced by temperature humidity and period of storage. Maize seeds are infected by three major pathogens namely fungi, bacteria, and viruses and that often reduce both the yield and the quality of grains as well as impair seed germination. As many as 112 diseases are known to occur in corn in corn-growing countries. Of all the diseases, more than 70 are seed-borne (USDA, 1960). A review of the literature shows that a number of fungi *viz*., *Alternaria alternata*, *Aspergillus* spp., *Bipolaris maydis*, *Fusarium moniliforme*, *Fusarium* spp., *Cephalosporium* spp., *Mucor* sp. and *Penicillium* spp., have been reported from maize seed (Tulin and Askun, 2006; Ehlam *et al*., 2015).

MATERIALS AND METHODS

The investigation was undertaken at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand, India during 2021-22. For this purpose, genetic identification was done to identify the fungal pathogen and Internal Transcribed Spacer (ITS) rDNA was employed to identify the fungal pathogen at the species level.

DNA extraction

For DNA extraction, the pathogen was grown on a PDA medium at 25 ± 2 °C for one week. Mycelia were harvested by filtering through Whatman filter Paper No. 1, washed repeatedly with distilled sterilized water to remove excess salts adhering to it. The DNA extraction was based on the cetyl trimethyl ammonium bromide (CTAB) method. Mycelium (1 g) was crushed in liquid nitrogen and transferred into 7.5 ml pre-warmed (65 °C) DNA extraction buffer [1 M TrisHCl (pH 8.0), 5 M NaCl, 0.5 M EDTA (pH 8.0) and 2% CTAB], mixed well and incubated in a water bath at 65 °C with gentle shaking for 45 min. An equal volume of chloroform: isoamyl alcohol (24:1%v/v) was added and mixed gently to denature proteins and centrifuged at 12,000 rpm for 15 minutes at 4 °C. The DNA was precipitated with 0.6 volume of chilled ethanol and 0.1 volume of 3 M sodium acetate (pH 5.2) and centrifuged at 18,514 g for 15 min. The pellets were washed twice with chilled 70% ethanol, dried at room temperature, and re-suspended in 100 µl sterile TE (10 mM Tris-HCl buffer and 1 mM EDTA-pH 8). Two microliter DNase-free RNase was added to dissolved DNA stock and incubated in a water bath at 37 °C for 45 min. followed by 65 °C for 10 minutes for enzyme inactivation. The DNA was quantified and qualified using NanoDrop Spectrophotometer and agarose gel electrophoresis. The sample was stored in a deep freezer at –20 °C temperature for long-term usage.

Sequencing of rDNA-ITS region

The dominant seed mycoflora was identified tentatively on a morphological level and was characterized at the molecular level using rDNA-ITS region sequencing. ITS 1

and ITS 4 primer pair were used for PCR amplification of the pathogen. The PCR reaction contained 50 ng genomic DNA, 1X PCR buffer (Thermo Scientific), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 µM of each primer, and 1.0 unit Dream Taq DNA Polymerase (Thermo Scientific) in a 50-µL reaction volume. The PCR program was: one cycle at 94 °C for 2 min, 35 cycles at 94 °C for 45 sec, 58 °C for 45 sec and 72 °C for 1 min and one cycle at 72 °C for 10 min and then held at 4 °C. The sequencing of the amplified product was carried out in an automated sequencer ABI 3730 genetic analyzer at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka, India.

Genetic data analyses

The sequence identity matrix of dominant mycoflora was generated using Bioedit Sequence Alignment Editor (Version 5.0.9) (Hall, 1999). After multiple alignments, phylogenetic analysis was done in MEGA 4.0 (Tamura *et al*., 2007) software using the default parameters of one character-based algorithm. The bootstrapped consensus phylogenetic tree was generated for each of these algorithms with 500 replication.

RESULTS AND DISCUSSION

The ITS region of fungal DNA was identified at the molecular level for the dominant fungus *Fusarium oxysporum* (rDNA).

DNA extraction and quantification

The CTAB DNA extraction methodology was used to isolate DNA, as described in the materials and methods section. The amount of DNA in the sample (605.82 ng/µl) was measured using NanoDrop Spectrophotometer at A260 nm/A280 nm and A260 nm/A230 nm. For further examination, samples with a high concentration of DNA and no RNA/protein contamination were chosen.

Polymerase chain reaction (PCR)

The ITS rDNA region was amplified using universal primer pairs ITS1 and ITS4 for PCR amplification. The primer combination of ITS1 and ITS4 produced an amplicon of 547 bp for *F. oxysporum*. The amplified product was analyzed on 1.8 per cent agarose gel.

Sequencing result of sample

The results were aligned and examined in the NCBI nucleotide BLAST to determine the similarity of newly sequenced samples to the GenBank databases, and they were matched at various global similarity levels. The ITS rDNA sequence of the sample was confirmed as *F. oxysporum* region of specific (Accession No. ON637065.1) covered the internal transcribed spacer-1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer-2, complete sequences and 28S ribosomal RNA gene, partial sequence. A BLAST search for similarities of *F. oxysporum* identification showed 99.16 that percentage of similarity of the isolates ranged from 98.36 to 99.16 per cent. Therefore, the species name assigned was according to the closest BLAST search. The pathogen of interest showed 99.16 per cent similarity with the already reported *F. oxysporum* (MN272279.1, MK751702.1, MF063025.1, KF534741.1, and JN585764.1) sequence.

Phylogenetic Analysis

The phylogenetic tree was constructed with nucleotide sequence from the sequenced ITS region of *F. oxysporum* (Fig. 1) and compared with other similar worldwide fungal isolates available in the NCBI database.

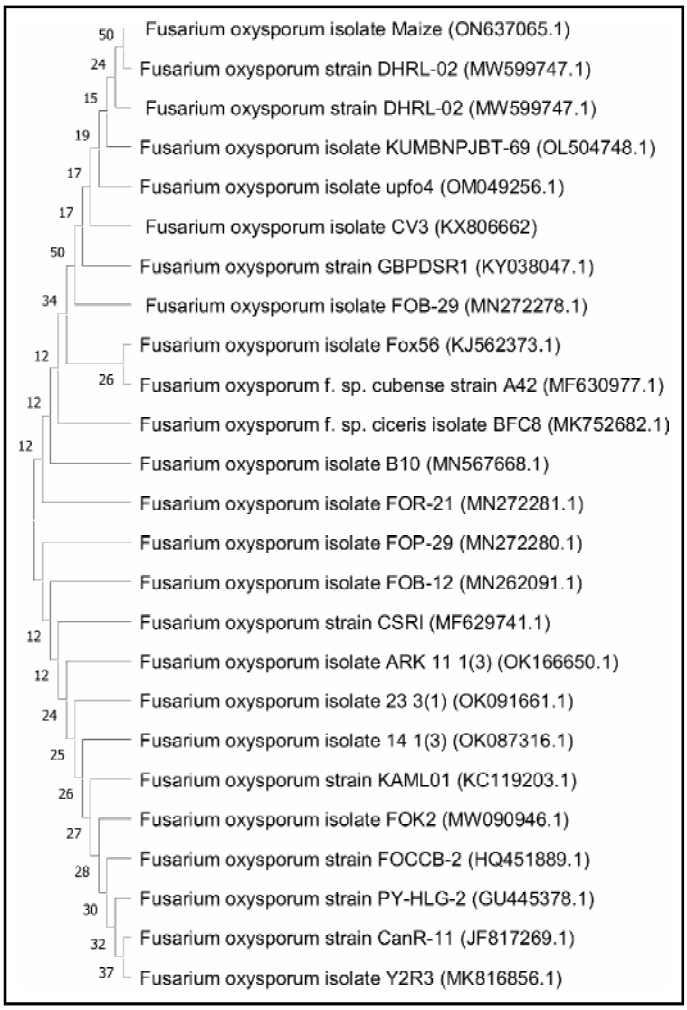

Fig. 1: Phylogenetic tree based on the sequence of rDNA ITS region of *Fusarium oxysporum*

CONCLUSION

The dominant mycoflora developed on maize seed samples during the seed mycoflora study was separately cultured on PDA and was used. Molecular identification through ITS rDNA region of dominant seed mycoflora isolated from maize cultivars revealed 99.16 per cent similarities with *Fusarium oxysporum* isolate.

ACKNOWLEDGEMENT

The first author is grateful to the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand for providing the necessary facilities.

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Online Manuscript No. 100667 recived on 25.8.2022
Manuscript accepted for publication on 18.11.2022

Journal of Eco-friendly Agriculture **18**(1): 188-192. 2023
doi: 10.5958/2582-2683.2023.00034.5

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Evaluation of solid and liquid substrates for mass proliferation of *Trichoderma* spp.

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ABSTRACT

Laboratory evaluation of different substrates for their suitability in mass production of five isolates of *Trichoderma* spp. revealed that the potato dextrose broth media providing largest population in isolate 1 (277.7 $\times 10^4$ cfu g^{-1}), isolate 2 (196.3 $\times 10^4$ cfu g^{-1}), isolate 3 (247.7 $\times 10^4$ cfu g^{-1}), isolate 4 (266.3 $\times 10^4$ cfu g^{-1}), and isolate 5 (237.3$\times 10^4$ cfu g^{-1}) after two months of storage proved the best. Different species' populations dropped gradually with the pace of loss varying with substrate. The lowest population of all the five species of *Trichoderma* was produced with rice straw.

Keywords: Evaluation, sabstrates, mass production, *Trichoderma* spp.

Different soil-borne and foliar pathogenic fungi harm economically significant agricultural, horticultural and decorative crop plants, resulting in crop losses of billions of dollars. The use of fungicides is now the most popular method of managing these diseases. There have been issues, too, including the emergence of pathogens that are resistant to fungicides and the failure of fungicides applied to seeds to shield mature plants' roots. The chemical control methods besides being expensive and non-remunerative, harmful to soil and human health, contaminate tood, feed and water and cause environmental pollution and its indiscrimenate use tends to development of resistance in plant pathogens against the chemical perticides used. Management of soil-borne diseases through chemicals being extremely challenging and expensive, the use of microbial antagonists appears to be the best alternative and environment friendly (Spiers *et al*., 2005; Bennet *et al*., 2006).

Trichoderma, a potent fungal biocontrol agent against a range, attracted considerable scientific attention (Rini and Sulochana, 2007). Its use in the management of several soil-borne diseases caused by pathogens like Rhizoctonia, Sclerotium, Fusarium, Pythium, and Phytophthora has been successfully utilised as fungal biocontrol agents in numerous crops (Cook and Baker, 1983). Trichoderma spp. are the subject of much investigation due to their widespread natural distribution, biocontrol potential against worm and fungal diseases, as well as their capacity to induce host defence (Haraman & Kubicek, 1998). Some deadly diseases including foot rot of black pepper, root rots and wilt of pulses, damping off, collar rots, and Fusarium wilt of horticultural crops are successfully controlled by bioagents like *T. harzianum*, *T. viride*, and *T. hamatum*. Successfull commercial production of *Trichoderma* depends on bio-efficacy, shelf life as well as the availability and affordability of a suitable substrate used in its multiplication. Maize, sorghum, pearl millet, wheat, wheat bran, wheat straw, used tea leaves, banana fruit peel, coffee husk, paddy straw, diatomaceous earth and earth granules soaked with molasses are only a few of the grains on which *Trichoderma* spp. have been produced (Zaidi and Singh 2004; Lewis and Papawizas, 1984). Utilizing easily accessible, affordable substrates, it is possible to economically produce large quantities of this antagonist. The majority of *Trichoderma* commercial formulations are based on inert material (talc), which solely serves as a carrier and bulking agent and has no effect on antagonist development or survival under storage. Biomass generation and preserving viability at the process conclusion are necessary for the creation of biological control agents (Adekunel *et al.,* 2001). Keeping in view the facts detailed as above, locally accessible organic substrates were evaluated for *Trichoderma* spp. mass growth and shelf life estimate.

MATERIALS AND METHODS

The study was conducted in Nauni, Solan, at the Dr. Y.S. Parmar University of Horticulture and Forestry's Department of Plant Pathology during 2015-16. Potato dextrose agar (PDA) medium was used to isolate the fungi *T. harzianum*, *T. viride*, and *T. hamatum* from soil samples taken from chrysanthemums. Dilution plate method was used to inoculate samples into plates, which were then incubated at 25°C for four days. The streaking method was used to purify

the fungal colonies, which were then cultured for 7-8 days at 25°C. Microscopic examination revealed *T. harzianum*, *T. viride*, and *T. hamatum* to be the green conidia producing fungal bodies that were chosen. On the basis of PDA slants, the culture was maintained.

Liquid media

Two liquid media viz. potato dextrose broth and malt yield extracts compsed with 250g of potato + 20g of dextrose + 1000ml of distilled water with the pH set at 6.5 and 4g malt + 4g yeast, + 10g dextrose, + 1000 ml distilled water, respectively were evaluated. Both the media were autoclave sterilized at 15 lb. pressure for 15–20 minutes at 121 °C.

Two bits of potential *Trichoderma* spp. measuring 4 mm each was placed in each flask containing 50 ml of liquid medium and incubated at 25± 1°C for six days. After reaching full growth, the population was counted every two months for a period of six months. At a dilution of 10^4, a colony count expressed as cfu (colony forming unit) was performed.

Solid media

Six solid media (wheat straw, farmyard manure, vermicompost, cocopeat and rice straw) gathered from two farms of department of Plant Pathology and department of Floriculture and Landscape Architecture) were evaluated. The substrates were cleaned of undesirable dirts like stones and foreign plant stuff. Concerning vermicompost and farmyard manure were added with 50 ml of distilled water for 30 minutes, while 1000 ml of distilled water was applied for two hours to humidify wheat straw, cocopeat and rice straw. After that, each bag was individually infected with four pieces (4mm) of prospective biocontrol agents, replicated three times and incubated in a BOD incubator at 25±1°C for eighteen to twenty days. The data on population counts (cfu $g^{-1} \times 10^4$) of all putative biocontrol agent from each solid medium were recorded after every two to eight months.

RESULTS AND DISCUSSION

For mass multiplication, *Trichoderma* species displaying the most antagonistic activity *in vitro* against the carnation wilt (*Fusarium oxysporum* f.sp. *dianthi*) and stem rot (*Rhizoctonia solani*) pathogens were chosen. The media were examined *in vitro* at ideal temperatures of 25 °C, pH 6.5, and incubation times of 2, 4, 6, and 8 months. The results are shown in table 1.

The growth and population count of *Trichoderma* species were supported by all the substrates. However, isolate 1 (Kandaghat) on potato dextrose broth (PDB) performed better reacheing to a maximum population count (CFU) (277.7) in contrast to wheat straw (172). The growth rate of all five isolates was higher in PDB after two months than in wheat straw in terms of time interval, and dropped subsequently in both the medium and the time interval. After eight months, the colony count in wheat straw decreased from 172 to 41 whereas it decreased from 277.7 to 97.67 in PDB. Molasses yeast extract (147.7), FYM (76.67), and vermicompost (67.67) were statistically equivalent in promoting growth, followed by cocopeat (53.00), mushroom compost (44.00), and rice straw (42.67).

PDB (97.67), wheat straw (41.7), molasses yeast extract (40.00), FYM (11.0), cocopeat (10.00), mushroom compost (10.00), rice straw (9.66), and vermicompost were found to have considerably lower colony counts after eight months (7.00). The findings also showed that isolate 1 (Kandaghat) out performed other isolates in potato dextrose broth and had the highest population count (277.7). Other isolates from the Rajgarh, Namhol, and Battu areas, including isolates 2 (196.3), 3 (247.7), and 4 (266.3), also demonstrated good growth and colony counts

***Trichoderma* spp. population count concerning natural antioxidants (crops)**

Impact of natural antioxidants generating crops in promoting growth and population count of *Trichoderma* spp. (Table 2) showed that, of all the tested crops, soybean (222.94) supported the highest population count, followed by rice (158.26), maize (156.56), ginger (128.22), turmeric (122.78), green tea (104.13), and sunflower (64.99) in terms of how well they performed on the substrate supplemented with natural antioxidants.

Regarding interactions between strains and antioxidant crops, it was found that Isolate 1 (Kandaghat) performed best, with colony counts reaching 247.7 after two months, followed by Isolates 2, 3, and 4 from Rajgarh, Namhol, and Battu regions. However, the same species performed better on rice and maize than other crops, and Isolate 4 from Battu recorded the lowest colony counts on sunflower (42) of all the isolates.

The optimal liquid media for *Trichoderma* spp. proliferation, according to Subash et al. (2013), is PDB. Our results are in line with Mamo and Alemu's (2012). Evaluation of various agricultural and industrial wastes utilising plastic bags, the wheat straw was observed generating more conidia from an economic and environmental standpoint. Wheat straw was listed as the most effective medium for *Trichoderma* spp. mass production by Chaudhari *et al.* (2010). Similar to this, Tewari and Bhanu (2004) tested several cellulosic substrates and found that wheat straw and paddy straw had the highest conidial production and the same colony

Table 1. Evaluation of different substrates for mass multiplication of *Trichoderma* spp.

Substrate	Population count (cfu/g × 10^4)						Overall Mean
	Interval	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	
A. Liquid media							
PDB	**2**	277.7 (2.44)	196.3 (2.29)	247.7 (2.39)	266.3 (2.42)	237.3 (2.37)	**245.06 (2.38)**
	4	197.3 (2.29)	157.7 (2.19)	189.7 (2.27)	206.0 (2.31)	186.3 (2.27)	**187.40 (2.27)**
	6	147.3 (2.16)	120.7 (2.08)	140.0 (2.14)	160.0 (2.20)	140.3 (2.14)	**141.66 (2.14)**
	8	97.67 (1.99)	66.33 (1.88)	69.33 (1.84)	77.67 (1.89)	71.00 (1.85)	**62.40 (1.89)**
Mean		**143.9 (1.78)**	**108.21 (1.69)**	**129.35 (1.73)**	**141.99 (1.76)**	**126.98 (1.73)**	
Molasses yeast extract	**2**	147.7 (2.16)	108.3 (2.03)	96.33 (1.98)	93.67 (1.97)	94.33 (1.97)	**108.0 (2.02)**
	4	76.33 (1.88)	60.33 (1.78)	57.33 (1.75)	49.00 (1.69)	50.00 (1.69)	**58.59 (1.75)**
	6	64.67 (1.81)	41.00 (1.61)	39.67 (1.59)	36.00 (1.55)	36.00 (1.55)	**43.46 (1.62)**
	8	40.00 (1.60)	18.33 (1.26)	18.33 (1.26)	17.00 (1.23)	13.00 (1.11)	**21.33 (1.29)**
Mean		**82.17 (1.86)**	**56.99 (1.67)**	**52.91 (1.64)**	**48.91 (1.61)**	**48.33 (1.58)**	
B. Solid media							
Wheat straw	**2**	172 (2.23)	115.7 (2.06)	104.3 (2.01)	102.3 (2.01)	103.3 (2.01)	**119.5 (2.06)**
	4	96 (1.98)	67.67 (1.83)	63.67 (1.80)	58 (1.76)	57.30 (1.75)	**68.52 (1.82)**
	6	70 (1.84)	46.00 (1.66)	39.00 (1.59)	36 (1.55)	30.33 (1.48)	**44.26 (1.62)**
	8	41 (1.61)	29.67 (1.47)	18.00 (1.25)	13.33 (1.12)	11.00 (1.04)	**22.6 (1.29)**
Mean		**94.75 (1.91)**	**64.76 (1.75)**	**56.24 (1.66)**	**52.40 (1.63)**	**50.48 (1.57**	
Vermicompost	**2**	67.67 (1.83)	66.33 (1.82)	60.00 (1.77)	58 (1.76)	59.33 (1.77)	**62.26 (1.79)**
	4	53.33 (46.91)	48.33 (44.05)	47.00 (1.67)	42 (1.62)	40.67 (1.60)	**46.26 (1.97)**
	6	19.00 (1.27)	16.67 (1.22)	19.33 (1.28)	25.33 (1.40)	24.00 (1.38)	**20.86 (1.31)**
	8	7.00 (0.84)	6.00 (0.77)	8.00 (0.90)	11.33 (1.05)	11.33 (1.05)	**8.72 (0.92)**
Mean		**36.75 (1.41)**	**34.33 (1.37)**	**33.58 (1.40)**	**34.16 (1.45)**	**33.83 (1.45)**	
FYM	**2**	76.67 (1.88)	66.00 (1.81)	56.33 (1.75)	72.00 (1.85)	68.00 (1.83)	**67.8 (1.82)**
	4	56.67 (1.75)	47.33 (1.67)	35.33 (1.54)	49.67 (1.69)	45.33 (1.65)	**46.86 (1.66)**
	6	23.33 (1.36)	26.00 (1.41)	17.33 (1.23)	15.67 (1.19)	13.67 (1.13)	**19.2 (1.26)**
	8	11.00 (1.04)	13.00 (1.11)	10.33 (1.01)	7.33 (0.85)	7.00 (0.84)	**9.73 (0.97)**
Mean		**41.91 (1.50)**	**37.33 (1.50)**	**29.83 (1.38)**	**36.16 (1.39)**	**33.5 (1.36)**	
Cocopeat	**2**	53.00 (1.72)	36.33 (1.56)	30.33 (1.48)	49.67 (1.69)	43.00 (1.63)	**42.46 (1.61)**
	4	43.33 (1.63)	29.00 (1.46)	26.33 (1.42)	36.33 (1.56)	30.33 (1.48)	**33.06 (1.51)**
	6	23.00 (1.36)	13.00 (1.11)	11.33 (1.05)	17.00 (1.23)	13.67 (1.13)	**15.6 (1.17)**
	8	10.00 (0.99)	6.33 (0.80)	5.66 (0.75)	8.00 (0.90)	6.00 (0.77)	**7.19 (0.84)**
Mean		**32.33 (1.42)**	**21.16 (1.23)**	**18.41 (1.17)**	**27.75 (1.34)**	**23.25 (1.25)**	
Rice straw	**2**	42.67 (1.63)	42.33 (1.62)	43.00 (1.63)	33.00 (1.51)	27.00 (1.43)	**37.6 (1.56)**
	4	35.33 (1.54)	33.00 (1.51)	31.67 (1.50)	21.00 (1.31)	18.00 (1.25)	**27.8 (1.42)**
	6	24.00 (1.38)	19.67 (1.29)	18.33 (1.26)	9.00 (0.95)	7.00 (0.84)	**15.6 (1.14)**
	8	9.66 (0.98)	8.33 (0.91)	9.00 (0.95)	4.66 (0.66)	3.00 (0.46)	**6.93 (0.79)**
Mean		**22.33 (1.11)**	**20.67 (1.07)**	**20.40 (1.07)**	**13.53 (0.89)**	**11.00 (0.80)**	
Mushroom compost	**2**	44.00 (1.64)	38.00 (1.57)	36.33 (1.56)	35.00 (1.54)	40.00 (1.60)	**38.67 (1.58)**
	4	38.00 (1.58)	29.00 (1.46)	27.67 (1.44)	30.00 (1.47)	30.33 (1.48)	**31.00 (1.49)**
	6	24.67 (1.39)	18.00 (1.25)	16.33 (1.21)	18.67 (1.27)	16.33 (1.21)	**18.80 (1.27)**
	8	10.00 (0.99)	8.00 (0.90)	6.00 (0.77)	5.00 (0.69)	7.66 (0.87)	**7.33 (0.84)**
Mean		**23.33 (1.12)**	**18.60 (1.04)**	**17.27 (1.00)**	**17.73 (0.99)**	**18.86 (1.03)**	

$CD_{0.05}$ Media (M)=0.001, Strains(S)=0.002, Interval(I)=0.001, M×S×I=0.006,
Figures in parenthesis are log transformed values

counts in 20 days old culture (4.95 and 4.86 ×10^8 g^{-1} powder, respectively). Additionally, wheat bran proved as excellent mass multiplication medium, according to Sargin *et al.* (2013). Pan and Das (2010) reported FYM and vermicompost also useful for preserving longer shelf lives of *Trichoderma* species. The eight agro-based substrates tested are readily accessible and inexpensive in the local area, however, the PDB-based substrates proved helpful in commercial-scale production with high-quality CFU counts, required for the use of bioagents for the treatment of plant diseases.

According to Sathiyaseelan *et al.* (2009), soybean oil has a longer shelf life than other cooking oils, which improves *Trichoderma* spp. survivability. According to

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00034.5

Table 2. Effect of natural antioxidants (crops) on population count of *Trichoderma* spp.

Natural antioxidant (crops)	Interval (Months)	Population count (cfu/g×10⁴) Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Overall Mean
Soybean (seeds)	**2**	247.7	243.7	242.3	196	185.0	222.94
		(2.39)	(2.38)	(2.38)	(2.29)	(2.26)	(2.34)
	4	226.3	220.0	221.3	172.7	166.7	201.4
		(2.35)	(2.34)	(2.34)	(2.23)	(2.22)	(2.29)
Mean		237	231.85	231.8	184.35	175.85	
		(2.37)	(2.36)	(2.36)	(2.26)	(2.24)	
Maize (grains)	**2**	197.7	122.7	139.7	163.7	159.0	156.56
		(2.29)	(2.08)	(2.14)	(2.21)	(2.20)	(2.18)
	4	176.0	99.33	120.0	141	136.3	134.52
		(2.24)	(1.99)	(2.07)	(2.14)	(2.13)	(2.11)
Mean		186.85	111.01	129.85	152.35	147.65	
		(2.26)	(2.03)	(2.10)	(2.17)	(2.16)	
Brown rice (grains)	**2**	161	128.3	198.3	119.7	184.0	158.26
		(2.20)	(2.10)	(2.29)	(2.07)	(2.26)	(2.18)
	4	147	116.7	173.7	96	163.7	139.4
		(2.16)	(2.06)	(2.24)	(1.98)	(2.21)	(2.13)
Mean		154	122.5	186	107.85	173.85	
		(2.18)	(2.08)	(2.26)	(2.02)	(2.23)	
Ginger (rhizome)	**2**	133.3	134.7	125.7	119.7	127.7	128.22
		(2.12)	(2.12)	(2.09)	(2.07)	(2.10)	(2.10)
	4	116.3	109.0	95.33	93.00	106.0	103.92
		(2.06)	(2.03)	(1.97)	(1.96)	(2.02)	(2.00)
Mean		124.8	121.85	110.51	106.35	103.92	
		(2.09)	(2.07)	(2.03)	(4.03)	(2.00)	
Turmeric (rhizome)	**2**	137.0	133.0	126.3	111.3	106.3	122.78
		(2.13)	(2.12)	(2.10)	(2.04)	(2.02)	(2.08)
	4	111.7	98.33	95.67	82.33	72.67	92.14
		(2.04)	(1.99)	(1.98)	(1.91)	(1.86)	(1.94)
Mean		124.35	115.66	110.98	193.63	89.48	
		(2.08)	(2.05)	(2.04)	(1.97)	(1.94)	
Green tea (leaves)	**2**	109.0	98.67	110	103	100	104.13
		(2.03)	(1.99)	(2.04)	(2.01)	(2.00)	(2.01)
	4	98.67	91.00	99.3	85	81.33	91.06
		(1.99)	(1.95)	(1.99)	(1.92)	(1.91)	(1.95)
Mean		103.83	94.83	104.65	94	90.66	
		(2.01)	(1.97)	(2.01)	(1.96)	(1.95)	
Sunflower (seeds)	**2**	117.3	69.67	51.00	42	45	64.99
		(2.06)	(1.84)	(1.70)	(1.62)	(1.65)	(1.77)
	4	96.33	55.67	33.67	25.67	23.33	46.93
		(1.98)	(1.74)	(1.52)	(1.40)	(1.36)	(1.6)
Mean		106.81	62.67	42.33	33.83	34.16	
		(2.02)	(1.79)	(1.61)	(1.51)	(1.50)	

$CD_{0.05}$ Media(M) = 0.004, Strain(S) =0.004, Interval(I) =0.002, M×S×I=0.02
Figures in parenthesis are log transformed values

Khandelwal *et al.* (2012), pulses, followed by rice and wheat, sustained the largest population count. Saju *et al.* (2002) employed wheat, grain of sorghum, wheat, pulse, and rice bran for mass production, which helped *Trichoderma* spp. in a lengthemy of its shelf life and giving better job in suppressing the *Rhizoctonia solani*-caused cowpea collar rot disease and encouraging plant development. Various cereal grains, including sorghum, millets, and ragi, were also employed by Jeyarajan (2006) for the industrial production of *Trichoderma* spp. after which the coated substrates were applied to seeds to cure them. According to Kaushal and Chandel (2017), wheat straw mixed with wheat flour and

natural antioxidant crops (soybean, maize, and rice) proved appropriate substrate for the mass production of *Trichoderma* spp., which is beneficial for the IDM programme. Coban and Sargin (2019) developed optimal conditions for the growth of *T. harzianum* EGE-K38 micropropagules in a bioreactor under a static liquid culture using Modified Czapec Medium (MCM), which resulted in the highest micropropagule count of 5.20.2x10 9 cfu/ml and the heaviest dry mycelia weight.

CONCLUSION

The study concludes that the potato dextrose medium prepared using 250 g of potato + 20 g of dextrose in 1000 ml distilled water of 6.5 pH produced larget population of all the five isolates of *Trichodarma viz*., 277 × 10^4 $cfug^{-1}$ in isolate 1 follwed by 266.3 × 10^4 $cfug^{-1}$, 247.7 × 10^4 $cfug^{-1}$, 237.3 × 10^4 $cfug^{-1}$ and 196.3 × 10^4 $cfug^{-1}$ in isolate 4, 3, 5 and 2, respectively and were recommarded for its use in commercial mass production.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Prof. & Head, Department of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, India, for providing essential facilities and the UGC for funding the project.

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Online Manuscript No. 99021 recived on 13.7.2022
Manuscript accepted for publication on 10.11.2022

Journal of Eco-friendly Agriculture **18**(1): 193-199. 2023
doi: 10.5958/2582-2683.2023.00035.7

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Evaluation of bio-control agents for the management of guava decline

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ABSTRACT

The area and production of guava has registered regular increase during last one decade due to its ever increasing demand and introduction of improved varieties. The wilt disease of guava caused by *Fusarium* spp. has been a major constraint and countrywide spread of the root-knot nematode, *Meloidogyne enterolobii* in recent years has made the issue extremely serious. Management of these soil borne pathogens has never been an easy task. Application of chemicals is not only expensive but also harmful to non-target organisms including human beings. Therefore, we evaluated the efficacy of *Trichoderma harzianum, Pochonia chlamydosporia, Purpureocillium lilacinum, Bacillus* sp., ICAR-FUSICONT and vermi compost alone and in combinations. All the treatments were effective in enhancing the plant growth and in suppressing pathogens. Bio-agents were more effective against *Fusarium oxysporum* when applied at temperatures optimum to their growth in comparison to higher. Pathogen activity was much higher at high temperature than at lower. Vermi compost + *Bacillus* sp. was found most effective against the nematode followed by *P. lilacinum, P. chlamydosporia and T. harzianum,* respectively.

Key words: Guava, decline, *Meloidogyne, Fusarium, Purpureocillium, Pochonia, Bacillus, Trichoderma*

Guava has been an attractive fruit crop for farmers as it starts bearing within two years of plantation, better returns compared to input cost, and provides income around seven months in a year. Its appreciable taste, rich nutritional status, over 90 per cent edible portion, digestion-friendly quality, and low price in comparison to other fruits attracts the consumers. Guava production was estimated at 4394 thousand MT from 299 thousand hectares during 2020-21 in India (NHB Database, 2021). The highest area (49.53 thousand hectares) and maximum productivity (18.79 MT ha^{-1}) were in Uttar Pradesh during 2017-18. Twenty-two states of our country have over 1000 hectares of area under guava, which indicates the wider adaptability of guava under different sets of climatic conditions. Almost all the states are recording continuous area expansion under guava.

Wilt is the most important soil-borne disease of guava throughout the world (Misra, 2017 and Shukla *et al.*, 2019). The guava wilt was first reported in Taiwan in 1926 and later in South Africa, Malaysia, India, and many more countries (Shukla *et al.*, 2019). In India, it was first reported in 1935 and since then a fair amount of research work has been carried out. Heavy yield and tree losses were reported till 1980 (Misra, 1996), but later disease management technology and farmers' efforts worked to reduce them to some extent. Several pathogenic fungi, *viz. Fusarium oxysporum, F. solani, F. chlamydosporum, F. proliferatum, Macrophomina phaseolina, Acremonium diospyri*, *Rhizoctonia bataticola, Gliocladium vermoesenii*, *Gliocladium roseum* syn *Clonostachys rosea* f.sp. *rosea*, *Verticillium albo-atrum*, *Nalanthamala psidii*, *Clitocybe tabescens*, *Myxosporium psidii*, and species of bacteria like *Pseudomonas* and *Erwinia psidii* have been found associated with wilt-affected plants in different guava growing countries (Misra, 2017) however, *Fusarium* spp. (*F. oxysporum* and *F. solani*) had most frequent association with guava wilt in India (Shukla *et al.*, 2019). *Fusarium* spp., the major cause of the disease, is a facultative saprophyte, which can survive in cultivated soil for several years in the absence of a suitable host. The fungus produces chlamydospores for long-term survival and numerous macro and microconidia for fast dissemination. The active growth and survival of the fungus depend upon the soil temperature, availability of nutrition, and moisture in the soil. The invasion of roots is made by the active hyphae growing in soil followed by infection and extensive colonization of root tissues. The infection proceeds gradually in the roots and may progress into the stem till the tree is wilted. The wilt-affected tree initially expresses nutrient deficiency symptoms (Misra, 2017), and later, yellowing or browning and curling of leaves have also been recorded. Depending on the weather factors, leaves may drop-down or stay on twigs and the fruits become mummified and remain hanging on trees for several months (Gomes *et al.*, 2011 and Misra, 2017). The infected roots and trunk of wilt-affected trees have dark brown or blackish discolouration.

Enough research has been carried out for the management of guava wilt since 1949 (Misra, 2017 and Shukla *et al*., 2019). The soil of the pit prepared for transplanting should be treated with 2 per cent formalin solution, be covered with polythene sheet for 3 days and then transplanting should be done 27 days after removing the polythene sheet (Misra, 2017). Application of green manure and organic matter in the rhizosphere of trees have been effective in decreasing disease incidence. Major pruning of affected trees, soil drench treatment with carbendazim at quarterly intervals for one year and spray with zinc sulphate and metasystox can revive wilt affected trees. Dwivedi (1993) has reported effectiveness of summer solarization using polyethylene sheet against wilt. Several more treatments like, neem cake + gypsum (Misra, 2017); growing intercrops of marigold and turmeric (Prasad *et al.*, 2003 and Anonymous, 2012); application of *Trichoderma harzianum* and *T. viride* in rhizosphere soil (Misra *et al.,* 2000, Dwivedi and Shukla, 2002 and Anonymous, 2020) have also been found effective against guava wilt. Rajan *et al.* (2005) have reported resistance in a cross, *Psidium molle* × *Psidium guajava* root stock, against wilt and utilized its root stocks for production of planting material.

Guava decline disease, caused by *Meloidogyne enterolobii,* has been recorded in recent past (Gomes *et al*., 2008, 2010, 2011, 2012, Poornima *et al*., 2016, Khan *et al*., 2019, Shukla *et al.,* 2018, 2019 and Singh, 2020). It has spread in most of the guava growing states of India during the last ten years and is now an established threat to guava production (Khan *et al*., 2019). Early age decline and wilting of guava plants has been due to the infection of *M. enterolobii* in the presence as well as absence of *Fusarium* spp. (Khan *et al*., 2019 and Shukla *et al*., 2019). Frequency of *Fusarium oxysporum* and *F. solani* has been higher in *Meloidogyne mayaguensis* and *M. enterolobii* infected plants as compared to root-knot nematode free orchards (Gomes *et al*., 2011, 2014 and Shukla *et al*., 2019). Studies carried out by Veloso *et al.* (2020) confirmed that the complex of *M. enterolobii* and *F. solani* or *Neocosmospora falciformis* was responsible for the guava decline in Brazil.

The nematode-infected plants initially express symptoms similar to the deficiency of nutrients and extensive rotting of infected roots (Gomes *et al.,* 2008). Infected trees may die in a shorter period and dry leaves stay attached to twigs if the infection is severe; otherwise, leaves may turn yellow or brown and shed gradually. The growth of infected trees becomes slow along with the reduction in number of flowers, fruits, and the size of fruits. Galls of 1 to 10 mm size develop on roots. The whole root system may get converted into galls in severely infected plants. Extensive rotting of nematode-infected roots occurs due to colonization by pathogenic fungi in fresh galls and by saprophytic fungi in old galls. Efficacy of nematicides *viz*., carbofuran and fluopyram is well known against root-knot nematode. *T. harzianum* application in the root zone soil of guava trees has been effective against decline (Jindapunnapat *et al*., 2013). Intercropping root-knot resistant cultivars of marigold, turmeric, and garlic with guava minimizes nematode infestation (Khan and Misra, 2003).

Till date efforts made for the management of guava wilt and decline have been separate for both the pathogens. Soil-borne disease management in perennial crops is always a challenge. We cannot solely depend on chemicals, therefore, evaluation of bio-control agents and organic products were necessary to manage the disease complex in guava.

MATERIALS AND METHODS

Experiments comprised of 18 treatments were carried out under a shade net house at ambient temperature from May to November 2020 and September 2020 to March 20, 2021 at ICAR-Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow (Table 1 and 2).

Procurement, maintenance and multiplication of cultures: The culture of *M. enterolobii* was maintained on roots of brinjal planted in sterilized pots and placed on a cemented platform under rain protected net house. The desired amount of second stage juveniles for experiments were procured by incubating egg masses under laboratory conditions. The cultures of *Purpureocillium lilacinum, Pochonia chlamydosporia,* and *Bacillus* sp. were procured from ICAR-National Bureau of Agricultural Insect Resources, Bengaluru. The cultures of *Fusarium oxysporum* f.sp. *psidii* and *Trichoderma harzianum,* isolated from roots of guava trees growing in orchards at Rehmankhera, Lucknow, were used in this study. The commercial formulation of ICAR-FUSICONT was procured from the bio-control laboratory of ICAR-CISH, Lucknow. The mass multiplication of bio-control agents was done on potato-dextrose broth medium and *Bacillus* sp. on nutrient broth medium.

Raising of seedlings: The substrate used for raising seedlings and for experiments, composed of sandy loam soil and FYM at the ratio of 8:2. The soil mixture was sieved and autoclaved for 2 hours at 121°C. The seeds of guava *cv.* Lalit, surface sterilized in 1.5% NaOCl for 3 minutes followed by several rinses in sterile water, were sown in 20 cm diameter earthen pots containing 2 kg substrate.

Application of treatments and inoculation: All the disease management treatments were applied to the substrate 7 days before transplanting of seedlings in both the experiments.

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00035.7

Fifteen-day-old cultures of fungal and 2-day-old culture of *Bacillus* sp. were applied @ 10^6 spores/CFU per kg of soil. ICAR-FUSICONT was applied @ 20 g kg^{-1} of soil and unsterilized vermi compost, @ 100 g kg^{-1} of soil. The juveniles were inoculated @ 2000 J2 per kg soil mixture and *Fusarium oxysporum* @ 10^6 CFU per kg of soil mixture at the time of transplanting of 45 days old seedlings.

Recording of data: The experiments were terminated about six months after inoculation. At termination, plants were uprooted, washed and data regarding shoot height (cm), shoot and root weight (g), root-knot index (0-4 scale), number of juveniles in soil, and colonization of roots by fungus were recorded. Development of galls on roots was rated on a 0-4 point scale, where 0- no galling, 1 = slight galling (1-25% roots colonized), 2 = moderate galling (26-50%), 3 = severe galling (51-75%), and 4 = very severe galling (76-100%). The postharvest nematode population in every replicate was determined separately by processing the 250 g soil using Cobb's sieving and decanting method followed by Baermann funnel technique. The isolated nematodes were counted twice under stereoscopic binocular microscope by filling nematode suspension in a one ml capacity counting slide and per g population was calculated. The colonization of roots by *Fusarium* was assessed by incubation of surface sterilized 25 pieces, representative of whole root system, on potato dextrose agar medium followed by counting the infected pieces.

Statistical analysis: We used a completely randomized block design with four replicates. The data were analyzed by ANOVA using OPSTAT software (Sheoran *et al.,* 1998), and the differences among the treatments were tested by the least significant difference test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Since guava is a perennial crop, long term experiments were conducted to assess long term effect of bio-control agents. Keeping in view the effect of temperature on plant growth and activities of microorganisms, the experiments were carried out during May to November (temperature ranged between 21-44 °C) and September to March (temperature ranged between 01-36 °C) under the same set of treatments.

The bio-control agents selected in this study, *Pochonia chlamydosporia, Trichoderma harzianum, Purpureocillium lilacinum* and *Bacillus* sp., are well known bio-control agents. *P. chlamydosporia,* an endophytic fungus, has been used in commercial products to control root-knot nematode infections, which also promotes plant growth and development (Philbrick, 2020, Monteiro *et al*., 2020 and De Souza *et al*., 2022). *Trichoderma* spp. are the most popular bio-agents used against nematode and fungal pathogens of guava and many more crops (Misra *et al.,* 2000, Dwivedi and Shukla, 2002, Dwivedi and Dwivedi, 2012, Jindapunnapat *et al.,* 2013, Naz *et al*., 2013 and Anonymous, 2020). *Purpureocillium lilacinum* parasitizes many root-knot and cyst nematodes observed on crops in many soil types and climates (Stirling and West, 1991). *Bacillus* sp. has bio-control potential through inhibitory activity against plant pathogens along with inducing systemic resistance in plants (Fira *et al*., 2018). ICAR-FUSICONT has shown great success against banana wilt (Damodaran *et al.,* 2019).

The maximum shoot height was recorded in T14 followed by T18, T11, T7, T10, T13, T16 and T12 (Table 1). The shoot height in T7, T10 and T11-14 was superior to T17, which indicated that these treatments were effective against the pathogens. The shoot weight was significantly high in uninoculated control as compared to different treatments and it was significantly improved in all the treatments in comparison to plants inoculated with both the pathogens. The results of plant height and weight indicated that most of the treatments were able to minimize the suppressive effect of pathogens on plant health (Table 1).

The root-knot index (RKI) was significantly reduced in six treatments (T6 to T11) as compared to control, which indicated that the combinations of all fungal bio-control agents with *Bacillus;* and *P. lilacinus* and *P. chlamydosporia* in combination with vermi compost were effective in suppressing the nematode infection (Table 1). Colonization of roots by *F. oxysporum* was not significantly different in fungus alone and nematode + fungus treatments, however it was proportionate to RKI, which indicated that root-knot infection enhanced the infection of fungus. No bio-control agent could suppress RKI significantly when applied alone but combinations worked.

Significantly superior shoot height was recorded in T6, followed by T4, T2 and T9-12 in comparison to the control plants inoculated with both the pathogens (Table 2). Shoot height in rest of the treatments was either lower or at par with the plants inoculated with both the pathogens. Shoot weight was significantly reduced in plants inoculated with nematode but not in fungus alone as compared to uninoculated control. When both the pathogens were inoculated together reduction in shoot weight was recorded significantly higher in comparison to either of the pathogen. Maximum shoot weight was recorded in vermi compost treated plants followed by *Bacillus* alone and combinations of bio-control agents with vermi compost. Maximum root weight was recorded in T14 followed by T12, T6, T8, T10 and T13. Root weight in rest of the treatments was recorded lower or at par with untreated inoculated plants (Table 2).

Development of galls on roots was best suppressed by T1-3 followed by T7, T4, and T6 treatments. RKI in rest of the treatments was recorded lower or at par with untreated inoculated plants. Number of juveniles recovered from soil ranged from 1.20 to 4.20 per g of soil, which was not important because it had no relation with other parameters. The colonization of roots by *Fusarium* was observed minimum in T1-3 followed by T4 and T6. In rest of the treatments it was recorded lower or at par with untreated inoculated plants (Table 2).

Table 1: Effect of various treatments on plant growth and pathogenic activities (May-September, 2020)

Tr. No.	Treatments	Shoot height (cm)	Shoot weight (g)	Root weight (g)	RKI (0-4 scale)	No. of J2 g^{-1} soil	% Root colonized by *Fusarium*
1	*M.e.* + *F.o.* + *P. lilacinum*	32.00 cd	4.26 e	7.84 ab	3.80 a	4.80 ab	26.67 a
2	*M.e.* + *F.o.* + *P. chlamydosporia*	31.40 cd	4.26 e	6.60 ab	3.84 a	4.60 ab	25.00 ab
3	*M.e.* + *F.o.* + *T. harzianum*	25.20 cd	3.58 e	3.64 c	3.60 ab	5.60 ab	24.00 ab
4	*M.e.* + *F.o.* + *Bacillus* sp.	30.60 cd	4.48 e	7.04 ab	3.90 a	5.00 ab	26.67 a
5	*M.e.* + *F.o.* + ICAR-FUSICONT	25.80 cd	3.24 e	4.28 bc	3.55 ab	5.40 ab	21.33 ab
6	*M.e.* + *F.o.* + Vermi compost	32.60 cd	4.34 e	3.54 c	1.78 b	4.40 ab	9.33 c
7	T1 + T4	40.80 bc	7.52 cd	2.86 cd	1.75 b	3.80 b	10.67 c
8	T2 + T4	21.00 d	3.90 e	3.24 cd	2.35 b	3.80 b	14.67 bc
9	T3 + T4	22.60 d	2.84 e	1.54 d	2.60 b	6.40 a	14.67 bc
10	T1 + T6	42.80 bc	11.82 bc	8.38 a	2.30 b	4.40 ab	13.33 bc
11	T2 + T6	46.40 b	11.10 bc	7.22 ab	2.43 b	3.60 b	18.67 b
12	T3 + T6	35.00 c	7.16 d	6.76 ab	3.50 ab	6.60 a	13.33 bc
13	T4 + T6	44.90 bc	9.54 cd	5.74 bc	3.20 ab	3.60 b	13.33bc
14	T5 + T6	61.80 a	17.10 a	7.18 ab	3.15 ab	4.40 ab	16.00 bc
15	*M. enterolobii* (*M.e.*)	50.10 b	12.60 b	5.40 bc	3.50 ab	3.60 b	0.00
16	*F. oxysporum* (*F.o.*)	45.30 bc	11.48 bc	4.00 c	0.00	0.00	17.33 bc
17	*M. enterolobii* + *F. oxysporum*	25.10 cd	4.52 de	2.20 cd	3.75 a	6.20 ab	13.33 bc
18	Uninoculated control	56.20 ab	17.48 a	6.00 b	0.00	0.00	0.00
	$CD_{0.05}$	10.47	2.35	1.99	1.11	2.44	7.36

Table 2: Effect of various treatments on plant growth and pathogenic activities (November, 2020-March, 2021)

Tr. No.	Treatments	Shoot height (cm)	Shoot weight (g)	Root weight (g)	RKI (0-4 scale)	No. of J2 g^{-1} soil	% Root colonized by *Fusarium*
1	*M.e.* + *F.o.* + *P. lilacinum*	27.92 bc	8.40 e	1.72 f	0.25 d	1.80 bc	1.33 c
2	*M.e.* + *F.o.* + *P. chlamydosporia*	29.90 ab	9.80 d	2.82 e	0.35 d	1.80 bc	1.33 c
3	*M.e.* + *F.o.* + *T. harzianum*	28.30 b	9.24 de	2.94 e	0.72 d	1.80 bc	2.67 c
4	*M.e.* + *F.o.* + *Bacillus* sp.	34.00 a	11.68 bc	3.60 d	1.90 bc	2.60 b	5.33 bc
5	*M.e.* + *F.o.* + ICAR-FUSICONT	17.70 c	3.81 h	1.38 f	2.80 a	3.60 ab	8.00 ab
6	*M.e.* + *F.o.* + Vermi compost	33.50 a	14.58 a	4.88 bc	2.10 bc	4.20 a	5.33 bc
7	T1 + T4	22.50 c	7.54 ef	3.32 de	1.50 c	1.20 c	6.67 b
8	T2 + T4	26.60 bc	10.00 c	4.84 bc	2.45 b	2.20 bc	8.00 ab
9	T3 + T4	30.00 ab	10.38 c	3.80 d	2.45 b	2.60 b	12.00 a
10	T1 + T6	31.20 ab	10.90 c	4.52 c	2.65 ab	2.40 bc	10.67 a
11	T2 + T6	28.90 ab	9.72 d	3.84 d	3.00 ab	2.40 bc	10.67 a
12	T3 + T6	32.40 ab	12.16 b	5.24 b	3.00 ab	4.20 a	10.67 a
13	T4 + T6	26.10 bc	7.72 ef	3.96 c	2.70 ab	3.20 ab	8.00 ab
14	T5 + T6	24.90 bc	10.84 cd	6.90 a	3.19 a	3.20 ab	12.00 a
15	*M. enterolobii* (*M.e.*)	29.10 ab	6.96 f	3.26 de	2.40 b	3.20 ab	0.00
16	*F. oxysporum* (*F.o.*)	29.20 ab	9.86 d	4.34 c	0.00	0.00	4.00 bc
17	*M. enterolobii* + *F. oxysporum*	23.50 bc	5.74 g	2.38 e	3.12 ab	3.00 ab	12.00 a
18	Uninoculated control	28.20 b	9.24 de	3.66 d	0.00	0.00	0.00
	$CD_{0.05}$	5.19	1.00	0.57	0.72	1.30	4.14

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00035.7

All bio-control used in this study have been found effective against *Meloidogyne enterolobii* on various crops including guava (Jindapunnapat *et al.* 2013 and Philbrick, 2020). Effectiveness of *Trichoderma* spp. is well proven against *Fusarium* spp. (Misra *et al.*, 2000, Dwivedi and Shukla, 2002, Dwivedi and Dwivedi, 2012 and Anonymous, 2020) but others play major role against the nematodes. Since, the introduction of *M. enterolobii* in India has made the guava wilt disease much more severe in the form of decline, we feel, if nematode is efficiently managed, the problem can be reduced. The results of this study agree with the findings of Naz *et al.* (2013) and Jindapunnapat *et al.* (2013). The recently developed commercial formulation, ICAR-FUSICONT for the management of banana wilt, was not found effective. The vermi compost was used as nutritional support and for testing of microbes it contain, against the pathogens of guava. Its application was found effective to an encouraging extent and when it was combined with bio-agents, it enhanced the efficacy of bio-agents under hot period. The study also indicated that the bio-agents should be applied preferably during the period of moderate temperature.

The bio-control agents were found less effective in first experiment laid-out during May however, in second experiment laid-out during September, better results were obtained in terms of effectiveness of the bio-agents. During the period of first experiment average weekly temperature ranged between 37.4°C (maximum) and 21.7°C (minimum) and probably higher temperature favoured nematodes more than bio-control agents. During the period of second experiment average weekly temperature ranged between 34.7°C (maximum) and 5.6°C (minimum) and most probably the lower temperature suppressed nematodes more than bio-control agents. The interpretation is supported by the reported preferred temperature ranges, 24-32°C for *Meloidogyne* (Diez and Dusenbery, 1989), 26–30°C for *P. lilacinum* (Senthilkumar *et al.*, 2020), 25-30°C for *T. harzianum* (Singh *et al.*, 2014 and Maurya *et al.*, 2017), 30-45ºC for *Bacillus* sp. (Berendsen *et al.*, 2016) and *Pochonia chlamydosporia (*Arevalo *et al.*, 2009 and Monteiro *et al.*, 2020).

T6 T8 T12 T17 T18

Fig. 1. Plant growth and nematode infection in different treatments (Tables 1 and 2)

While looking beyond data and statistics, the close observation of roots of plants treated with T6, T8 and T12 indicated that the roots available at the time of inoculation were severely infected with the nematode but further growth of roots was rarely infected (Fig. 1). This indicated that the bio-control agents could not suppress the nematodes for few days after inoculation and they could cause the infection, but later generations of nematodes were suppressed. It gives better hope of success in bio-management of guava decline.

CONCLUSION

The bio-control of guava decline can be achieved by using either of bio-agents, *Pochonia chlamydosporia, Purpureocillium lilacinum, Trichoderma harzianum,* alone or in combinations with *Bacillus* sp. and vermi compost. Temperature may play major role in survival and activity of bio-agents, therefore, should be applied at optimum temperature.

ACKNOWLEDGEMENTS

The authors are highly thankful to the Director, ICAR-Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow for providing facilities and the Director, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru for providing cultures and financial support under AICRP-BC.

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Manuscript recived on 16.10.2022

Manuscript accepted for publication on 20.11.2022

Journal of Eco-friendly Agriculture **18**(1): 200-202. 2023
doi: 10.5958/2582-2683.2023.00036.9

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Research Article

Rice false smut disease: An emerging threat to the paddy growers in Tamil Nadu

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ABSTRACT

Rice false smut (*Ustilaginoidea virens* (Cooke) Takahashi) is an upcoming major disease in India. False smut is recently emerging as a major Rice disease that was previously considered to have a negligible impact. It is found in two different stages sexual and asexual and both spores can infect the spikelet and lead to the formation of a smut ball of rice grain. In Tamil Nadu, the overall disease incidence (percentage of false smut-infected tillers) varied from 3 to 65 per cent and the overall mean severity of the disease ranged from 29.08 to 36.61 per cent. The highest mean disease severity was observed in the Thanjavur district (36.61%) followed by the Trichy district (30.67%), least mean disease severity (29.08%) was observed in the Ariyaluru district. Among different blocks, the mean disease severity of false smut disease ranged from 4.39 to 58.52 per cent of which the highest mean disease severity was recorded from the Thirumanur block (58.52%) followed by Thanjavur (56.70%), Thuraiyur (54.08%) and least disease severity of 4.39 per cent were recorded from Andhanallur block of Trichy district.

***Key words*:** Rice, False smut, *Ustilaginoidea virens,* Tamil Nadu

Rice is one of the most important and widely cultivated food crops in the world. Among various biotic stresses affecting rice production, false smut caused by *Ustilaginoidea virens* (Cooke) Takahashi is known to cause significant quantitative and qualitative losses in grain yield, and epidemics of false smut disease of rice were reported in Tamil Nadu in India and later in many countries of the world (Rush *et al*., 2000; Singh and Pophaly, 2010). It is a common grain disease of rice around the world. The disease was first reported from Tinnevelli in Tamil Nadu, India by Cooke in 1878 (Ou, 1972). There are reports of severe incidences causing severe yield losses because many smutted balls replace healthy grains (Singh and Dube, 1978). The main reason for losses being incited is that the fungus attacks the panicles. About 15-20 per cent of losses have been reported by different workers from different provinces (Singh, 1998). Disease incidence was found to be 2-75 per cent for Northwest states and 5-85 per cent from the southern state of Tamil Nadu (Ladhalakshmi *et al*., 2012). Agarwal and Verma (1978) reported losses varying from 7 to 75 per cent in India. In Uttar Pradesh, yield losses of up to 44 per cent were observed by Singh and Dube (1978). In recent years, it has emerged as the most devastating grain disease in the majority of rice-growing areas of the world. In India, the disease has been observed in severe form since 2001 in major rice-growing states, *viz*., Haryana, Punjab, Uttar Pradesh, Uttaranchal, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Jharkhand, Gujarat, Maharashtra, Jammu & Kashmir and Puducherry (Dodan and Singh, 1996; Mandhare *et al*., 2008). The fungus transforms individual grains of the panicle into greenish spore balls of velvety appearance. The spore. balls are small at first and grow to a size of two inches or more in diameter. They are smooth and yellow, covered by a membrane. Later, the membrane bursts, and the colour of the ball becomes orange/yellow. When cut open, the ball is white in the center with three outer layers (Sciumbato and Street, 2000). The fungus forms chlamydospores and sclerotia late in the season which fall in the soil and may survive the winter. Sclerotia germinate to produce ascocarp-containing ascospores, which are the primary source of infection in rice plants, whereas secondary infection may come from airborne chlamydospores. In infected plants, the mature head is replaced by velvety smut balls which are globose and yellowish-green. When the smut balls burst open, powdery dark green spores are released (Atia, 2004). Although the disease has been very well studied in countries like China, Japan, Egypt, and the USA during the last 8-9 years, its disease cycle is still a mystery, and primary infection is a controversy. Very scanty and sporadic information is available in India. The life cycle of the pathogen is still required to be correlated with the disease cycle of the false smut pathogen. Reports showed that rice false smut pathogen could produce two kinds of mycotoxins, namely Ustiloxins and Ustilaginoidins (Zhou *et al*., 2012). This disease results

in yield loss, contaminated rice grains, and even more important, generating toxins poisoning humans and domestic animals (Koiso *et al*., 1994; Zhou *et al*., 2012). The present study was undertaken to fill up this research gap and to update our existing knowledge on the false smut pathogen in India.

MATERIALS AND METHODS

An intensive roving survey was conducted during the year 2021 to assess the severity of false smut in Tamil Nadu districts which included Trichy, Thanjavur and Ariyalur, and recorded the false smut incidence at dough and grain maturity stages in farmer's fields. In each district, four talukas were selected and from each block, three villages were selected. In each village, three farmer's fields were selected randomly. In each field, three random plots of 1 sq m were selected and observations on the number of infected tillers/ m^2 and a number of smut balls/infected panicles were recorded. Percent infected tillers and percent infected grains were calculated using the following formula (Mandhare *et al*., 2008)

$$\text{Percent infected tillers} = \frac{\text{Total number of infected tillers/m}^2}{\text{Total number of tillers/m}^2} \times 100$$

$$\text{Percent smutted grains} = \frac{\text{Number of diseased grains/panicles}}{\text{Total number of grains/panicles}} \times 100$$

Disease severity was calculated using the following formula (Singh and Dube, 1978).

Disease severity (%) = Infected tillers (%) x Smutted grains (%)

RESULTS AND DISCUSSION

An intensive roving survey was conducted in 2021 to assess the incidence of false smut in different districts of Tamil Nadu *viz*., Trichy. Thanjavur and Ariyalur districts and recorded the false smut severity at panicle emergence to grain maturity stages. Observations such as infected tillers, grains infected, and disease severity were recorded and presented in Table 1.

During the survey, we collected the false smut balls and isolated the pathogen. *Ustilaginoidea virens* is a very slow-growing fungus and appeared as a tiny white colony 7 days after inoculation. A single well-isolated colony of the fungus was picked up and maintained as a pure culture. *Ustilaginoidea virens* began to grow in culture medium producing a white colony, flat or raised with slight undulations, mycelium fluffy, compact and leathery. Microscopic observations showed that the mycelium is septate and single oval shape conidia.

Table 1. Survey for the severity of false smut of rice in Tamil Nadu

District	Blocks	Infected Tillers (%)	Smutted Grains (%)	Disease Severity (%)
Trichy	Andhanallur	3.25	1.35	4.39
	Lalgudi	7.90	4.00	31.60
	Manachanallur	7.50	4.35	32.63
	Thuraiyur	10.30	5.25	54.08
	Mean	7.24	3.74	30.67
Thanjavur	Budalur	4.30	3.00	12.90
	Papanasam	7.50	4.35	32.63
	Thiruvaiyuru	8.50	5.20	44.20
	Thanjavur	10.50	5.40	56.70
	Mean	7.70	4.49	36.61
Ariyalur	Thirumanur	10.45	5.60	58.52
	Ariyalur	7.50	4.00	30.00
	Sendurai	4.57	2.30	10.51
	T. Palur	5.40	3.20	17.28
	Mean	6.98	3.78	29.08

Results from Table 1 indicated that the false smut incidence noticed in all surveyed locations ranged from 4.39 to 58.52 per cent and the overall district mean severity of the disease ranged from 29.08 to 36.61. The infected tillers and infected smutted grains varied from 3.25 to 10.50 and 1.35 to 5.60 per cent, respectively.

Among the three districts, the highest mean disease severity was observed in the Thanjavur district (36.61%) followed by Trichy district (30.67%), least mean disease severity (29.08%) was observed in the Ariyaluru district.

Among different blocks, the mean disease severity of false smut disease ranged from 4.39 to 58.52 per cent of which the highest mean disease severity was recorded from the Thirumanur block (58.52%) followed by Thanjavur (56.70%), Thuraiyur (54.08%) and least disease severity 4.39 per cent was recorded from Andhanallur block of Trichy district.

In the Trichy district, the mean disease severity ranged from 4.39 to 54.08 per cent. The highest mean disease severity was recorded in the Thraivur block (54.08%) followed by Manachanallur (32.63%), Lalgudi (31.60%), and the least disease severity was recorded in the Andhanallur block (4.39%).

In Thanjavur district, the mean disease severity ranged from 12.90 to 56.70 per cent. The highest mean disease severity was recorded in the Thanjavur block (56.70%) followed by Thiruvaiyuru (44.20%), Papanasam (32.63%), and the least disease severity was recorded in the Budalur block (12.90%).

Similarly, in Ariyalur district, the mean disease severity ranged from 10.51 to 58.52 per cent. The highest mean disease

severity was recorded in the Thirumanur block (58.52 %) followed by Ariyalur (30.00 %), T. Palur (17.28 %) and the least disease severity was recorded in the Sendurai block (1051 %).

This is mainly due to the change in weather, rainfall, varietal profile, and other geographical features of the respective location. Previous investigations have also reported the variation in disease severity within a region (Ladhalakshmi *et al.*, 2012; Singh *et al*., 2014; Kumar, 2015; Muniraju, 2017; Shivamurthy, 2017 and Banasode and Hosagoudar 2020). Similarly, Singh, 2019, conducted a survey for the prevalence of false smut of rice in October-November, 2019 from selected rice-growing districts of Punjab and Haryana. Among all the districts surveyed, the highest incidence (75 %) of false smut of rice was recorded in Phagwara tehsil of Kapurthala district in Punjab and Rohtak tehsil of Rohtak district (55 %) in Haryana. Incidence ranged between 10 to 75 per cent in surveyed districts of Punjab and Haryana. During 2021 Chaudhari *et al*. (2021) surveyed 11 locations, Jamalapada recorded the maximum percent disease incidence *i.e.*, 12.15, 10.06 and 11.11 during 2017, 2018 and mean of two years, respectively. The maximum average smutted ball per panicle recorded was 2.80 per cent in Jamalapada, during 2017 and 2.52 per cent in Khatal, during 2018, while the mean maximum average smutted ball was 2.63 per cent in Jamalapada. The maximum disease severity index, observed in Jamalapada was 34.02, and 24.65 per cent during 2017 and 2018, respectively with a mean of 29.15 per cent.

Therefore, it is concluded that the cultivation of susceptible cultivars, uncertified seeds, high input cultivation, continuous monocropping, and favorable weather conditions are very much essential for higher false smut disease severity.

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Online Manuscript No. 100545 recived on 16.8.2022
Manuscript accepted for publication on 18.11.2022

Journal of Eco-friendly Agriculture **18**(1): 203-205. 2023
doi: 10.5958/2582-2683.2023.00037.0

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Short communication

Impact of biofertilizers and organic manures on Vase life of Gladiolus (*Gladiolus grandifloras* L.) cv. Pusa Suhagan

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ABSTRACT

A field trial laid out in a randomized block design with 7 treatments and three replications at central Agricultural University, Imphal, Manipur during 2021-22 showed that the treatment incorporating 75 per cent vermicompost + azotobacter @ 2 g corm^{-1} + PSB @ 2 g corm^{-1} recorded minimum transpiration to water uptake ratio (1.34), maximum no of open florets (95.78), maximum days for opening of top most floret in the spike (11), wilting of top most floret in the spike (15.17), water uptake upto senescence (88 ml), longevity of each open florets (days) and diameter of the full open florets (cm) and vase life (9.33).

Key words: Gladiolus, Azotobacter, PSB, vermicompost, FYM, Poultry manure, Vase life

Gladiolus, believed to be the "queen of bulbous flowers", has gained popularity in many parts of the world owing to its supreme beauty. The name gladiolus originated from a *Latin* word '*gladius*', which means sword because of its sword shaped foliage. It is also called "sword lily". Gladiolus is also known as "Corn flag" in Europe because *Gladiolus illyricus* is found as wild weed in corn field. Gladiolus, a member of family Iridaceae and originated from South Africa, is a prominent bulbous cut flower plant. It is one of the most cultivated and economically important ornamental flower for trading in India stands at 4th place in the international trade, after rose, carnation and chrysanthemum.

The continuous and unbalanced use of conventional fertilizers leads to decreased nutrient uptake efficiency of plants resulting in decreased crop yield. It also causes serious threat to soil health. To cope with all these problems a cheaper, better and safer way is necessary in order to improve soil fertility status, maximize the agricultural productivity with minimum eco hazards. All these criteria can be achieved through application of organic manures and biofertilizers for restoring the soil fertility and improve physio-chemical and biological properties of soil. Few workers reported that the production of quality flowers with maximum yield in gladiolus is possible under organic cultivation.

The field trial was laid out in the experimental field of Central Agricultural University, Imphal, Manipur, located at latitude of 24° 81′ N with longitude measuring 93° 89′ E at an elevation of 784 m above mean sea level in a Randomized Block Design during 2021-22. The experiment comprised of 11 treatments and replicated thrice. The treatments were T1: Control (60:150:150) T2: FYM @ 12 t ha^{-1}, T3: vermicompost @ 2 t ha^{-1}, T4: poultry manure @ 2 t ha^{-1}, T5: 75 per cent FYM + azotobacter @ 2 g corm^{-1} + PSB @ 2 g corm^{-1}, T6: 75 per cent vermicompost + azotobacter @ 2 g corm^{-1} + PSB @ 2 g corm^{-1}, T7: 75 per cent poultry manure + azotobacter @ 2 g corm^{-1} + PSB @ 2 g corm^{-1}. The soil of the experimental plot was found to be acidic with pH of 5.7 with clay soil type and organic carbon content of 1.59 per cent. The soil has an available nitrogen, phosphorus and potassium of 428.58 kg ha^{-1} (medium), 36.27 kg ha^{-1} (medium) and 249.35 kg ha^{-1} (medium). The plot size was 2.4 m^2 (2.4 × 1 m) and 30 plants were planted in each plot. The corms were sown at a spacing of 40 cm × 20 cm. The data collected for various parameters were subjected to a statistical analysis by Fisher's method of analysis of variance for testing the significance of the treatments effect and their interpretation of data as given by Gomez and Gomez (1984).

The findings pertaining to the vase life of the flowers are given in Table 1. The treatment T_6 (75% vermicompost + azotobactor @ 2 g corm^{-1} + PSB 2 g corn^{-1}) recorded the lowest transpiration to water uptake ratio (1.34), the maximum water uptake by the flower (88 ml), the highest percentage of opened florets (95.78%), the highest percentage of opened florets (95.78%), highest rachis length (68.60 cm) and the maximum flower. It diameter of 7.93, 7.77 and 7.73 cm in 2nd, 4th and 6th florets, respectively also recorded maximum longevity of 2nd, 4th and 6th florets (4.67, 4 and 3.83 days, respectively), days to opening of top floret (11 days), of top

Table 1: Effect of biofertilizers and organic manures on vase life parameters

Treatment Combinations	Transpiration to water uptake ratio	Total water uptake (ml)	No. of open florets (%)	Longevity of florets			Diameter of florets (cm)			Days to taken opening of top floret	Days taken to wilting of top floret	Vase life
				2nd floret	4th floret	6th floret	2nd floret	4th floret	6th floret			
T1: Control (60:150:150)	1.41	76.17	90.84 (9.53)	3.33	3.00	2.83	7.53	7.22	6.63	9.67	12.60	7.17
T2: FYM @ 12t/ha	1.42	75.67	88.67 (9.42)	3.33	3.00	2.67	7.23	6.93	6.20	9.33	12.00	6.67
T3: Vermicompost @ 2t/ ha	1.39	79.67	91.62 (9.57)	3.67	3.33	3.17	7.63	7.23	6.67	10.00	13.33	7.33
T4: Poultry manure @ 2t/ha	1.41	76.67	90.68 (9.52)	3.33	3.00	2.83	7.47	7.17	6.57	9.50	12.67	7.00
T5: 75% FYM + Azotobacter @ 2g/corm + PSB @ 2 g/ corm	1.36	85.33	93.73 (9.68)	4.33	3.67	3.50	7.70	7.47	7.00	10.67	14.67	8.00
T6: 75% vermicompost + Azotobacter @ 2g/corm + PSB @ 2 g/ corm	1.34	88.00	95.78 (9.79)	4.67	4.00	3.83	7.93	7.70	7.37	11.00	14.83	9.33
T7: 75% Poultry manure + Azotobacter @ 2g/corm + PSB @ 2 g/ corm	1.37	84.00	92.56 (9.62)	4.00	3.33	3.33	7.67	7.40	6.73	10.33	14.00	7.67
S.Ed(±)	0.01	2.98	0.09	0.43	0.27	0.33	0.14	0.17	0.16	0.385	0.376	0.56
C.D. (p= 0.05)	0.03	6.50	0.20	0.94	0.59	0.81	0.31	0.38	0.34	0.839	0.820	1.22

floret (14.83), and maximum vase life was (9.33 days). The increase in water uptake and low transpiration to water uptake in T_6 may be attributed to more sink potential arising out of more number of florets spike^{-1} as well as larger size of florets. Increase in post-harvest attributes of cut spikes due to application of integrated nutrient sources could be attributed due to the presence of ethylene inhibitors or due to the presence of cytokinins in organic sources which delayed the senescence of flowers. These results are in corroboration with the findings of Chaudhary *et al*., (2013) in gladiolus, Amos *et al.* (2021) in statice. The highest number of opened florets seen in treatment T_6 might be due to sufficient accumulation of carbohydrates, enhancing petal movement. Similar results were found by De *et al*. (1999) in cut rose. Diameter of the florets is maximum in T_6, this could be the reason that the vermicompost contain GA, that low GA concentration might help in increasing the levels of reducing sugar in stems and flower heads of cut spikes increased the osmotic potential and turgidity of flower heads and hence facilitates the expansion of flowers (Emongor, 2004 and Saeed *et al*., 2014). Maximum longevity of each open floret, days to opening of top floret and wilting of top floret seen in T_6 might also be due to a fact that higher water absorption maintained better water balance and flower freshness thus saving the florets from early wilting. These results were in accordance with Varu and Barad (2010) and Mukesh *et al*., (2007). Increment in vase life might be due to reduction in ethylene synthesis which has a harmful effect for flower life. Biofertilizers and vermicompost regulate nutrient uptake process and prolonged vase phenomenon. Our findings are in harmony with Singh *et al*., (2014), Kumar, (2014) and Khan *et al*. (2009) in tulip.

CONCLUSION

It was concluded that the application 75 per cent vermicompost + azotobacter @ 2 g corm^{-1} + PSB @ 2 g corm^{-1} is the best to increase vase life in gladiolus.

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Online Manuscript No. 100525 recived on 12.8.2022
Manuscript accepted for publication on 12.11.2022

Journal of Eco-friendly Agriculture **18**(1): 206-207. 2023
doi: 10.5958/2582-2683.2023.00038.2

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Short communication

Increasing mustard productivity through cluster frontline demonstration in the Mahendragarh district of Haryana, India

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ABSTRACT

Cluster Frontline Demonstrations (CFLDS) of improved technologies of crop production conducted with mustard variety RH-725 in 172 ha areas by KVK, Mahendragarh district in Haryana during 2019 and 2020-21 revealed that the yield under demonstration plots were higher, 19.26 and 24.63 q ha^{-1} as against 17.10 and 21.10 q ha^{-1} under farmer's practices. The demonstration plot also showed higher gross and net returns ₹ 86975 and ₹ 108945 ha^{-1} and ₹ 52415 and ₹ 76050 ha^{-1} in the respective years than those in the local check plots. The B:C ratio in demonstrated plot i.e. 2.16 and 3.31, respectively was also higher than the local check plot. The results clearly indicate the impact of adoption of improved technology on productivity of mustard over the existing practices.

Keywords: Cluster Frontline demonstration, Technology, Intervention, Economic, Impact and mustard

India is one of the leading oilseed producing countries in the world. Oilseeds form the second largest agricultural commodity after cereals. Mustard is the second important edible oilseed crop after groundnut, meeting the fat requirement of about 50 per cent population in all the northern states. It plays an important role in the oilseed economy of the country. It is grown as oilseed crop as well as condiment and for their medicinal uses. Edible oils are next to food grains in Indian diet. The young plants used as vegetable provide enough sulphur and minerals in the diet. Its oil is used for softening leather and in the preparation of hair oils, medicines, soaps, greases. The oil cake is used as a cattle feed and manure. Oilseeds are rich source of energy and nutrition. Edible oil and oil meals have an important role to play in relieving malnutrition and caloric nutrition of human being and animals (Hedge, 2004). India's contribution to the world oilseed production is still very low (9.54%) because of extremely low productivity of different oilseed crops. It is the major source of income especially to small and marginal farmers in rainfed area of country. India produced 8.43 million metric tons of rapeseed and mustard in the year 2018-19. The area under rapeseed and mustard in India is 6.63 million hectares, with a productivity of 1270 kg ha^{-1} during 2018-19 (FAOSTAT).

Cluster frontline demonstrations on mustard were conducted under the guidance of scientist at farmer's field with integrated use of resources available at field with improved technology to increasing the productivity of mustard and get higher returns. It is now widely accepted fact that training to farmers and farm women increases the technical knowledge regarding package of practices of mustard crop. KVKs are playing a vital role across the farming community in imparting knowledge in distinguish field also. The main objective of Cluster Front-Line Demonstrations is to demonstrate newly released varieties and its management practices in the farmers' field under different farming situations with field constrains. Cluster Frontline Demonstrations are conducted in a block of two or more in order to have better impact of the demonstrated technologies on the farmers' and field level extension functionaries. Productivity of crops per unit area could be increased by improved practices in a systematic manner along with high yielding varieties (Ranawat *et al*., 2011 and Rai *et al*., 2016).

The demonstrations were conducted at farmer's field during *Rabi* seasons during 2019-20 and 2020-21 covering 60 and 112 ha in eight clusters in Mahendragarh District of South Haryana. A group of 144 and 280 farmers' were selected on the basis of information collected before laying out of demonstration to know the technology adoption gaps. The improved practices of mustard production were followed in demonstration plot. The improved package of practices followed by farmers under CFLD included use of quality

Table 1: Effect of improved practices of CFLDs on mustard yield at farmer's field

Crop	Season	No. of demonstration	Area (ha)	Average yield (q ha^{-1})		Yield increase (%)
				Improved practice	Farmer's practice	
Mustard	Rabi 2019-20	144	60	19.26	17.10	12.63
Mustard	Rabi 2020-21	280	112	24.63	21.10	16.72
	Average	-	-	21.95	19.10	14.68

Table 2: Effect of improved practices of CFLDs on economics of mustard at farmer's field

Season	Gross cost of cultivation (Rs ha^{-1})		Gross returns (Rs. ha^{-1})		Net returns (Rs. ha^{-1})		B:C ratio	
	IP	FP	IP	FP	IP	FP	IP	FP
Rabi 2019-20	34560.0	31870.0	86975.0	77420.0	52415.0	45550.0	2.52	2.43
Rabi 2020-21	36610.0	32895.0	126420.0	108945.0	89810.0	76050.0	3.45	3.31
Average	35585.0	32382.5	106697.5	93182.5	71112.5	60800.0	2.985	2.87

IP- Improved practices and FP- Farmer practices

seed (RH-725), treatment of seed with bavistin 2 gm kg^{-1}, sowing of seeds in the last week of September to 20th October at 30 cm (row to row), 15-20 cm (plant to plant) distance, use of (80 kg N + 30 kg P_2O_5 + 20 kg K_2O + 40 kg S + 25 kg $ZnSO_4$ ha^{-1} + bio-fertilizers, weed management through pre-emergence application of pendimethalin 30 EC 3.3 l ha^{-1} followed by manual weeding at 30 days after sowing, light irrigation before flowering and after pod development (If no rainfall), foliar application of bavistin (0.1%) at 45 and 60 DAS and application of mencozeb @ 1.5 kg ha^{-1} at 45 and 60 DAS. Plot size for demonstration was of 0.4 ha. The yield and returns of demonstration plots were compared with local check yield in the way suggested by Yadav *et al*. (1999). The following formula was used for the calculation of benefit: cost ratio.

$$\text{Benefit: cost ratio} = \frac{\text{Average gross/returns (Rs./ha)}}{\text{Average cost of cultivation (Rs./ha)}}$$

The data on the effect of improved practices on yield presented in table 1 showed that the demonstration plots recorded increased yield during respective years. The average yield of mustard in two years was 21.95 q ha^{-1} against 19.10 q ha^{-1} in local check plot (Table 1). The results are in consonance with Chaudhary *et al*., 2018.

Effect of improved practice on returns (table 2) showed higher gross and net returns in demonstration plots than under the farmer's practice. Gross and net returns obtained from demonstration plots (₹ 106697.5 & ₹ 71112.5 ha^{-1}) were more than under farmer's practice (₹ 93182.5 & ₹ 60800.0 ha^{-1}) during 2019-20 and 2020-21, respectively.

CONCLUSION

The higher B:C ratio in demonstration plot could be attributed to higher yield in demonstration plots than under farmer's practice. It was found that gaining knowledge regarding improved technology through training, demonstration, group meeting, mela, field days and visits proved useful in dissemination of technologies from Lab to field.

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Online Manuscript No. 97556 recived on 8.4.2022
Manuscript accepted for publication on 12.11.2022

Journal of Eco-friendly Agriculture **18**(1): 208-210. 2023
doi: 10.5958/2582-2683.2023.00039.4

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Short communication

Seed preference and biology of rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in stored green gram

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ABSTRACT

The study on biology revealed that the duration of egg laying, incubation, larval period, pupal period and total life cycle of *T. castaneum* lasted for 6.40, 6.60, 30.80, 5.20 and 49.0 days, respectively. The best preferred the groundnut (ICGS-76) seed the most damaging 10.01 per cent of the seeds and leading to 1.21 per cent loss in weight under storage while coriander (local cultivar) was found the least preferred with 0.33 per cent seed damage and 0.01 weight loss.

Keywords: Larval period, pupal period, total life cycle, seed damage, weight loss

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is an important pest of stored product, particularly the food grains in tropics (Howe, 1956). It generally prefers the seed embryo but may also feed on whole kernels. It is a polyphagous and cosmopolitan pest having extreme adaptation, supported by the presence of a cryptonephridial kidney-like organ allowing its survival under extreme low humidity conditions (Richards *et al*., 2008). It damages the grain by reducing the mass and/or volume, physiological quality, germination capacity and increasing the temperature and water content of the grains (Padin *et al*., 2002). The beetles in large numbers causes the flour to becomes greyish and discoloured. The pungent and disagreeable odour given off by the insect scent glands into the flour, gives it a disgusting taste and odour (Good, 1936). In comparison to temperate countries where it only survives temporarily, it is especially more prevalent in warmer climates (Hill, 2002).

T. castaneum is one of the most cosmopolitan pests of stored products with a polyphagous feeding habit (Bachrouch *et al*., 2010) and attacking widespread variety of stored products and their by-products. Wheat flour is regarded as most preferred substrate (Saim and Meloan, 1986). Its attacks on peanuts and groundnuts have also been reported (Redlinger and Simonaitis, 1977) and other cereals like rice, maize and sorghum etc. and their flours (Ajayi and Rahman, 2006). Beans, dried flours, pasta, spices, cocoa beans and chocolate, dried pet food, dried specimens in museum, yam and cassava (Dharmaputra, *et al*., 1991), Pearl millet (Lale and Yusuf, 2000), wheat grain (White and Lambkin, 1988), Soya bean meal (Cox and Simms, 1978) have also been reported as hosts for red flour beetle. Keeping in view the economic importance the pest has, its biology in stored green gram along food preference with different range of seeds *viz*., pea (Makhayat mubi), Pea (Makuchabi), Pea (Rachana), Soy bean (DSb-32), Green gram (Pant M-6), Groundnut (ICGS-76), Groundnut (ICGS-76), Broad bean (local cultivar), Lentil (PL-4), Maize (chakhao-chujak), Wheat (Sonalika) and Coriander (local cultivar) was studied.

Biology of *T. castaneum* was studied with 100 g of green gram seeds taken in 5 plastic jars and released with a pair of adults in each. These jars were covered with muslin cloth and tied with rubber bands to prevent the adults from escaping and were kept in B.O.D incubator at 33± 2^{0}C temperature. Seeds were examined daily for the observations during developmental period like egg, larval, pupal and adult (Male & Female) periods. Duration of all the life stages as well as the total developmental period (egg to adult) on green gram seeds was recorded. Twenty pairs of newly emerged male and female beetles were released over fifty grans healthy seeds of pea (Makhayat mubi), Pea (Makuchabi), Pea (Rachana), Soy bean (DSb-32), Green gram (Pant M-6), Groundnut (ICGS-76), Groundnut (ICGS-76), Broad bean (local cultivar), Lentil (PL-4), Maize (chakhao-chujak), Wheat

(Sonalika) and Coriander (local cultivar) obtained from Department of Genetics and Plant Breeding and Department of Entomology, College of Agriculture, Central Agricultural University, Imphal kept in 3 replications and were kept at 33± 2°C temperature in B.O.D incubator to assess the better preference to different crop seeds. Observations on percent damaged grains/seeds by counting the total number of infested/damaged grains and healthy grains in each replications of different seed was recorded after 90 days treatments. Loss in grains/seeds weight was calculated by considering initial and final weights after removing all the insect parts and different insect stages without any loss in grain particles. Percentage weight loss will be calculated based on formula suggested by Boxall (1986).

$$\text{Percentage weight loss} = \frac{(\text{UNd}) - (\text{DNu})}{\text{U}(\text{Nd} + \text{Nu})} \times 100$$

Where,

U = Weight of undamaged grain

Nu = Number of undamaged grain

D = Weight of damaged grain

Nd = Number of damaged grain

Mean values of the data were subjected to statistical analysis after suitable transformation and significance between the treatments was tested by using analysis of variance.

The mean egg laying (oviposition) period and incubation period lasted for 6.40 and 6.60 days. Pires *et al.* (2019) reported egg incubation period of 4.20± 0.4 days (Table 1). The larvae passed through 6 to 7 instars. The first instar larvae right after hatching measured nearly 1 mm was creamy white in colour, thin, slender and wiry in appearance (Photo 1). The matured one measured about 5 to 6 mm. Larvae are cylindrical in shape with slight brownish body dorsaly and whitish ventraly covered with small fine hairs. It tooks 30.80 days to pupate. Larval duration of 37. 22 days reported by Sundar *et al.* (2021) supports the current study.

Final instar larvae undergo pupation and form naked pupae without any cocoon. Pupation usually takes place outside the seeds. Sexual differentiation can be done in the pupal stage. Female pupae can be differentiated from male pupae by the presence of genital lobes at the tip of the abdomen on ventral side. Shameema *et al.* (2021) distinguished female pupae from the male by the presence of papillae at the tip of its abdomen. The mean pupal period lasted for 5.20 days.

Newly emerged adult beetle are light in colour and become reddish-brown later. They are oblong in shape. Females are larger than the male. They have a pair of 11 segmented club shaped antennae. Their forewings are sclerotized and fly very rarely. Adults emits stinky odour making the infested grain unsuitable for human consumption. The female, live longer (31.6 days) than the males (13.4 days). The total life cycle from egg laying to adult emergence observed was of 49.00 days at 33±2°C. Inglis (2013) reported 40-42 days of life cycle at temperature ranging from 20°C- 30°C. The duration of *T. castaneum* life cycle under all favourable conditions during summer is relatively of shorter.

The results on seed preference based on extent of seed damage and weight loss recorded after 90 days exposure (table 2) exhibited significant differences between mean seed

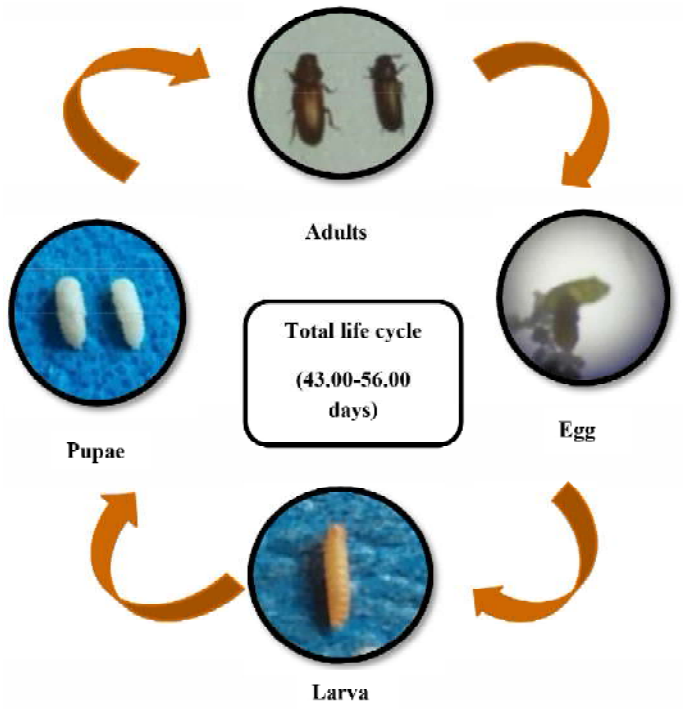
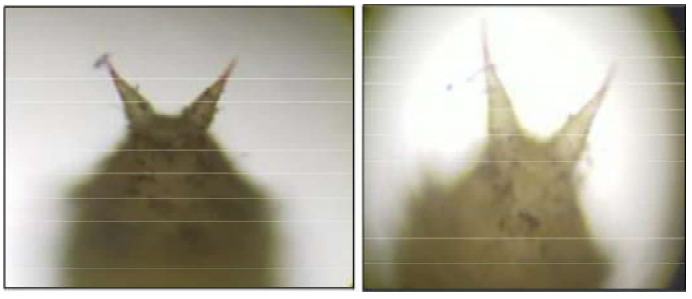


Photo 1. Complete life stages of *T. castaneum*



Male pupae Female pupae

Photo 2. Genital lobes of pupae *T. castaneum*

Table 1. Duration of different life stages of *T. castaneum* on green gram

Different life stages	Average days taken ± SD (n=5)
Oviposition period	6.40 ± 2.30
	(4.00- 9.00)
Incubation period	6.60± 2.07
	(4.00- 9.00)
Larval period	30.80± 4.32
	(25.00- 37.00)
Pupal period	5.20± 0.84
	(4.00- 6.00)
Total life cycle	49.00± 4.76
	(43.00- 56.00)
Adult longevity- Male	13.40± 2.07
	(11.00- 16.00)
Female	31.60± 3.36
	(28.00- 36.00)

Figures in the parentheses indicates the range of five observations

Table 2. Assessment of food preference of *T. castaneum* on different seeds

Variety	*Per cent seed damage (%)	*Per cent weight loss (%)
Pea (Makhayat mubi)	1.91 (1.55)	0.32 (0.90)
Pea (Makuchabi)	1.74 (1.49)	0.31 (0.90)
Pea (Rachana)	0.94 (1.20)	0.07 (0.76)
Soy bean (DSb-32)	1.01 (1.22)	0.20 (0.84)
Green gram (Pant M-6)	2.28 (1.67)	1.15 (1.29)
Groundnut (ICGS-76)	10.01 (3.24)	1.21 (1.31)
Broad bean (local cultivar)	5.05 (2.35)	0.13 (0.79)
Lentil (PL-4)	0.40 (0.95)	0.05 (0.74)
Maize (chakhao-chujak)	3.04 (1.88)	0.47 (0.97)
Wheat (Sonalika)	3.65 (2.04)	0.37 (0.93)
Coriander (local cultivar)	0.33 (0.91)	0.01 (0.71)
SE(d)	**0.09**	**0.08**
CD(p=0.05)	**0.19**	**0.16**

Figures in the parentheses are square root transformed values
*Data represented in the table are mean of three replications

damage and weight loss. Groundnut (ICGS-76) seeds recording highest mean per cent seed damage (10.01%) and 1.21 per cent loss in seed weight was the most preferred seed against all other evaluated.

CONCLUSION

The study concludes that total life cycle of *T. castaneum*, from egg laying to adult emergence, completed in 49.00 days. Among different crop seeds tested, groundnut (ICGS-76) seed was most preferred by *T. castaneum*.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Entomology, College of Agriculture, Central Agricultural University, Imphal for providing all the facilities.

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Online Manuscript No. 100469 recived on 6.8.2022
Manuscript accepted for publication on 14.11.2022

Journal of Eco-friendly Agriculture **18**(1): 211-213. 2023
doi: 10.5958/2582-2683.2023.00040.0

ISSN : 2229-628X (print)
e-ISSN : 2582-2683 (online)

Short communication

Critical stage analysis for Alternaria leaf spot diseases against black gram under artificial inoculations

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ABSTRACT

The present investigation was carried out to evaluate the critical stage for *Alternaria* leaf spot against black gram under artificial inoculation in pot culture. The study revealed that the susceptibility of the black gram plants to *Alternaria* leaf spot increased with the increase in age and it reached to maximum at the age of 40 DAS with PDI 57.60. Minimum disease was observed in the plants ageing 10 DAS with PDI 16.80. In general, as black gram plants grew older, they became more susceptible to *A. alternata.* The study showed no severe infection of *Alternaria* leaf spot in younger leaves and plants of black gram.

Keywords: Blackgram, Alternaria leaf spot, plants age, critical stage, date after sowing.

Blackgram (*Vigna mungo* (L). Hepper), also known as Urdbean, is a self pollinating diploid grain legume (2n=2x=22) belonging to the *Leguminaceae* family and have a genome size of 560 mb (Arumuganath and Earle 1991). It is one of the most economically important legume crop of South-East Asia widely adapted to both semi-arid and subtropical areas. During *kharif* season, it is grown as a sole crop or mixed with maize, sorghum, pearl millet and pigeon pea. In *rabi* and *zaid* (summer) seasons, it is cultivated as a pure culture. Urdbean contains approximately 25-28 per cent protein, 1.0-1.5 per cent oil, 3.5 - 4.5 per cent fibre, 4.5-5.5 per cent ash and 62-65 per cent carbohydrates on dry weight basis. High values of lysine make urdbean an excellent complement to rice in terms of balanced human nutrition. Being short duration and photo, thermo insensitive, it is considered as an excellent crop for intensification and diversification.

Blackgram crop is affected by several fungal and viral diseases of which *Alternaria* leaf spot is a very important limiting its productivity. The disease, incited by *Alternaria alternata,* cause reduction in annual productivity to the extent of 20-30 per cent (Trivedi *et al.,* 2013) and is posing an increasing threat to legume crops around the globe including India, causing leaf spot and other diseases on over 380 plant species (Laemmlen, 2001). Among them, the pathogen has been reported to attack cauliflower, cabbage, chili, papaya, wheat, rice sorghum etc. causing leaf spot, rots and blight diseases (Rathod, 2012).

Alternaria is a dictyosporic genus of the family *Pleosporaceae*, order *Pleosporales*, fungi imperfecti. Nees first established the genus, *Alternaria*. *Alternaria alternata* (originally *A. tenuis*) as type species is reported in 1817 (Rotem, 1994). Fries (1832) had proposed the *A. alternata* as *Torulaalternata* Pers. The reasons why the specific epithet '*alternata*' should be used instead of the more commonly accepted one '*tenuis*' was clearly stated by (Simmon, 1986). Keissler (1912) and Srinath and Sarwar (1965) had given the morphology of *A. alternata* and *A. tenuissima*. The species of *Alternaria* may be segregated into several groups according to catenulation (number of spore in the chain). *A. alternata* belong to *Longicatenatae* in which conidia appear in long chains of approximately 10 spores or more, either beakless or with very short beak. *A. alternata* has been reported on at least 115 host plants from 43 families (Neergaard, 1945).

Black gram plants were raised by transplanting in 30 cm earthen pots having Soil: FYM (3:1) mixture from Rajsthan College of Agriculture. A pot experiment was laid out in completely randomized design (CRD) with each five replications. Five pots for each plant age group were maintained. Black gram plants of different ages were achieved by staggered sowing at 10 days interval so as to obtain 10, 20, 30, and, 40 days old plants for simultaneous inoculation at one time. Five pots for each plant age group (having 5 plants each) were maintained in cage house and high humidity was maintained throughout the disease development period by regular irrigation. Observations for

disease severity of *Alternaria* leaf spot were recorded when plants reach the physiological maturity using 1-5 disease rating scale given by Sangeetha and Siddaramaiah, 2007 (Table 1).

Table 1. Disease rating scale given by Sangeetha and Siddaramaiah, 2007.

Scale	Description of the symptoms
1.	Small irregular spots covering <5% leaf area.
2.	Small irregular brown spots with concentric rings covering 5.1-10% leaf area.
3.	Lesions enlarge, irregular brown with concentric rings covering 10.1-25% leaf area.
4.	Lesions coalease to form irregular and appears as a typical blight symptom covering 25.1-50% leaf area.
5.	Lesions coalease to form irregular and appears as a typical blight symptom covering >50% leaf area.

The per cent disease index (Wheeler, 1969) was calculated as

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all individual disease rating}}{\text{Total No. of plants assessed} \times \text{maximum rating}} \times 100$$

The study on effect of plant age on development of *Alternaria* leaf spot was carried out on susceptible local land race of pot grown blackgram plants. Four different stages were spray inoculated simultaneously at one time with *A. alternata*, using inoculum density of $1x10^3$ conidia. Disease severity was recorded using 1-5 disease rating scale as discussed in material and methods and the per cent disease index (PDI) was calculated. The results revealed that the susceptibility of blackgram plants to *Alternaria* leaf spot gradually increased with increasing plant age. Here younger plants exhibited more susceptibility than older plants.

The data (Table 1) revealed that the blackgram plants inoculated at different plant age *i.e.,* 10, 20, 30 and 40 days after sowing showed *A. alternata* infection. The susceptibility of blackgram plants to *Alternaria* leaf spot gradually increased with increase in plant age. Maximum *Alternaria* leaf spot was observed in the plant ageing 40 DAS with PDI 57.60 and was found to exhibit significantly highest susceptibility over other tested plant ages. It was followed by plants ageing 30 DAS with PDI 43.80, next to follow 20 DAS with PDI 24.40 and plants with age of 10 DAS were found to exhibit the significantly lowest susceptibility against *A. alternate* with PDI 16.80. The most susceptible age of blackgram was 40 days old plant. At this age, plants showed maximum PDI of 57.60 (Table 2 and Fig. 1).

Saad and Hagedorn, 1969 also observed that the proneness to susceptibility to attack of *A. alternata* increased

Table 2: Effect of plant age in relation to *Alternaria* leaf spot severity under artificially inoculated condition on organically pot grown black gram plants

Plant age (DAS)	Per cent disease index (PDI*)
10	16.80
	(24.20)
20	24.40
	(29.60)
30	43.80
	(41.44)
40	57.60
	(49.37)
SEm±	0.458
C.D.5%	1.373
C.V.%	2.87

*Mean of five replications
DAS: Days after Sowing
Figures in parentheses are arcsineÖper cent angular transformed values.

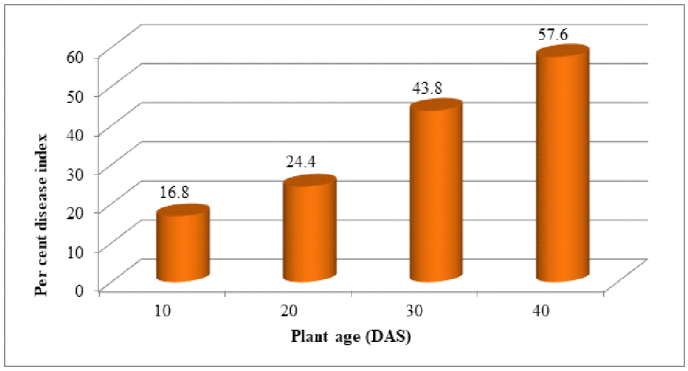

Fig. 1: Effect of plant age in relation to *Alternaria* leaf spot severity under artificially inoculated condition on organically pot grown black gram plants.

with increase the age of host plant. During the anthesis and seed filling stage of sunflower plants were more vulnerable to infection by *A. alternata* (Kumar *et al.,* 1976 and Islam *et al.,* 1976). Mishra *et al.* (2002) reported that 8 week old sunflower plants had maximum susceptibility to infection of *A. alternata*. Radish plants were more vulnerable at mature stage as compare to young stage to infection by *A. raphani* observed Sangwan *et al.* (2002). The results in present investigation are in tune with the aforesaid reported review.

CONCLUSION

It was concluded that the susceptibility of the Blackgram plants to *Alternaria* leaf spot increased with increase in age and reached at its maximum at the age of 40 DAS with PDI 57.60. Minimum disease was observed in the plants ageing 10 DAS with PDI 16.80.

Journal of Eco-friendly Agriculture **18**(1) 2023
doi: 10.5958/2582-2683.2023.00040.0

ACKNOWLEDGEMENT

The authors sincerely thanks the Dean and Head, Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur for providing necessary facilities to conduct the investigations.

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Online Manuscript No. 97103 recived on 10.3.2022

Manuscript accepted for publication on 16.7.2022

Author Index

STATEMENT ABOUT OWNERSHIP AND OTHER PARTICULARS ABOUT JOURNAL OF ECO-FRIENDLY AGRICULTURE

FORM IV

(See Rule 8)

1.	Place of Publication	Lucknow – 226 016 State- Uttar Pradesh, India
2.	Periodicity of its Publication	Biannual
3.	Printer's Name Address	Army Printing Press 33, Nehru Road, Sadar Cantt, Lucknow - 226 002
4.	Publisher's Name Address	Doctor's Krishi Evam Bagwani Vikas Sanstha, A-601, Indira Nagar, Lucknow - 226 016, UP, India
5.	Editor's Name Nationality Address	Dr. (Mrs). Neelima Garg Indian Head of Post-Harvest Management Division ICAR-CISH, Lucknow (U.P.)
6.	Name and addresses of individual who own the newspapers and partners or shareholders holding more than one per cent of the total capital	Dr. R.P. Srivastava, General Secretory, Doctor's Krishi Evam Bagwani Vikas Sanstha, A-601, Indira Nagar, Lucknow - 226 016 Registration No. 1-131380

I, Dr. R.P. Srivastava, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dated: 01.01.2023

Sd./ Dr. R.P. Srivastava

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Doctor's Krishi Evam Bagwani Vikas Sanstha

(Doctor's Agricultural and Horticultural Development Society)
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Application for the Fellow of Doctor's Agricultural and Horticultural Development Society (FDAHDS)

1. Name in full
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2. Date of Birth
3. Field of specification
   (i) Major discipline (section) :
   (ii) Subject of specification :
4. Present Position/Designation:
5. Address : (a) Office : Tel. No. :
   Fax No. :
   E-mail :
   Mobile :
   Address :
   (b) Residential : Tel. No. :
   Mobile :
   Address :
6. Academic record and professional attainments:-

Exam/Degree/Diploma	Institution/Board/University	Year	Distinction
Higher Secondary			
Graduation			
Post graduation			
Ph. D			

7. Service record: -

Position Held	Organization	Institution & Place of Posting	Period (From-To)	Duration (Year, months)

8. Significant Achievements of the Nominee
9. Awards & Honors, including Scientific Leaderships
   (i) International Awards
   (ii) National Honor/Awards
   (iii) Other Honors/Awards
   (iv) Scientific Leadership
10. Publications

List below 20 best research publications in the descending order of Journals of impact and high standing (as identified by the NAAS): indicating (i) name of author(s), (ii) year, (iii) title, (iv) name of journal, volume no. and page nos.

S.No.	List of Publications*	NAAS Journal ID	NAAS Rating

*Note: *List of Journals identified by the Academy and their assigned weightage is available on Academy web site http://www.naasindia.org*

11. Please attach additional list of publication
12. Papers published in Journal of Eco-friendly Agriculture

I certify that the information given in the form is correct.

Date: Signature of Nominee

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Journal of Eco-Friendly Agriculture Vol. 18, No. 1, January, 2023