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3. Palmer, J.D. 1992. In Cell Organelles (R.G. Herman, Editor) Springeru Verlag, Vienna, 99.133.
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Website : www.pernatrut.in, E-mail : annalsofhorticulture@gmail.com

RNI No. UPENG/2008/30221

PRINT ISSN 0974-8784

ONLINE ISSN 0976-4623



HTHS

Annals of Horticulture

Volume 19 Issue 1 June, 2026

(A peer-reviewed or refereed journal)



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Size : Full Page Rs. 2000.00 (India) US\$ 50.00 (Abroad), Half Page Rs. 1000.00 (India) IS\$ 25.00 (Abroad)

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R.N.I. No. UPENG/2008/30221

PRINT ISSN 0974-8784

ONLINE ISSN 0976-4623

Annals of Horticulture

(A peer-reviewed or refereed journal)

Volume 19 Issue 1 June, 2026



HI - TECH HORTICULTURAL SOCIETY

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ANNALS OF HORTICULTURE

Volume 19 (1)

2026

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EVALUATION OF GLADIOLUS VARIETIES FOR ECONOMICALLY IMPORTANT CHARACTERS

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Abstract

The present experiment was carried out to evaluate fifteen varieties of gladiolus (*Gladiolus hybridus* Hort.) for different morphological parameters during the year 2019-2020. Analysis of variance revealed wide and significant variations for most of the characters. The variety Praha took minimum days to sprouting of corm (5.17 days). Minimum days to spike emergence (66.67 days) and opening of first floret (86.21 days) were recorded in variety Gunjan. Plant height was recorded maximum in variety Shabnam (105.47 cm), whereas spike length (89.93 cm) and rachis length (55.64 cm) was found maximum in Essley Blue. Maximum floret size was found in variety Essley Blue (9.82 cm). Number of florets opened at one time was found maximum in variety Nova Lux (8.47). Shabnam showed maximum duration of flowering (17.98 days) and number of florets per spike (14.22). Suchitra recorded maximum vase life of 17.33 days. Maximum number of corms per plant was found in variety Viola (2.27). Corm diameter (6.77cm) and corm weight (100.00 g) was recorded maximum in the variety Praha. However, number of cormels per plant (41.97) and weight of cormels per plant (13.42g) was found maximum in variety Essley Blue.

Key words: Gladiolus, floret, spike, rachis, corm, vase life.

INTRODUCTION

Gladiolus (*Gladiolus hybridus* Hort.), also known as sword lily, is one of the most popular bulbous flowers grown in many parts of the world and it has a long and noble history (Singh, 2006). It belongs to the family Iridaceae (Prasad and Kumar, 2003). It is native of South Africa and was introduced into India during 19th century. It is grown all over the world for its majestic flower spikes, which bear attractive florets, having distinct patches

or markings of various colours and colour combinations with excellent vase life and hence regarded as “Queen of the bulbous plants” (Priyanka *et al.*, 2018 and Bhattacharjee and De, 2005). Commercially, it is popular as a cut flower. However, it is also used for making bouquets, flower arrangements, interior decoration and mass planting in beds in gardens (Abbasi *et al.* 2005). In gladiolus, a wide range of variation exists with respect to shape, size, growth habit

and flowering behaviour. Evaluation is the first important step in any breeding programme. It is the basic tool for assessing the genetic variability present in any crop species, which could be exploited for its commercialization. As in flower crops, there is always a demand for novel types with high yielding genotypes. Hence, it becomes mandatory to evaluate new varieties for various desirable traits and finally to recommend the suitable types for commercial cultivation in a particular region. In view of this, the present study was undertaken to study the performance of gladiolus varieties in Bundelkhand region.

MATERIALS AND METHODS

The present experiment was conducted at Instructional Farm, College of Horticulture, Banda University of Agriculture & Technology, Banda, Uttar Pradesh during Rabi season 2019-20. The University is located at 24°53'-25°55' N latitudes and 80°07'-81°34' E longitudes and an altitude of 128 m above mean sea level. The experimental material comprised of fifteen varieties of gladiolus. The experiment was laid out in randomized block design (RBD) with three replications. Field was properly ploughed and brought to a fine tilth by ploughing and harrowing. Thereafter, field was leveled properly and then plots were prepared according to the layout plan. Healthy and uniform sized corms (4 to 5 cm diameter) of fifteen varieties of gladiolus were taken and cleaned by removing the dried husk present on them. Thereafter, corms were dipped in captan (0.2%) solution for 25-30 minutes as preventive measure for Fusarium wilt. These corms were planted at a spacing of 30 x 20 cm in each row at a depth of 5-6 cm. Light irrigation was given immediately after planting. Well decomposed farm yard manure was added at the rate of 5 Kg/m² at the time of land preparation (Arora, 1992). Recommended dose of NPK (40:20:20 g/m²) was applied in the

form of Urea, Single Super Phosphate and Muriate of Potash respectively. Full dose of potassium and phosphorus was added as basal application at the time of field preparation. Nitrogen was added in two split doses first half dose was applied at 3-leaf stage and remaining half dose was applied at 6-leaf stages (Mukherjee *et al.*, 1994). All the recommended cultural operations were strictly followed during the experiment. Observations were recorded on five randomly selected plants in each replication for various vegetative, flowering, post harvest and corm characters. The observed data were subjected to statistical analysis and interpretations were made according to results presented in Tables 2 to 3.

RESULTS AND DISCUSSION

Analysis of variance revealed significant variation among different varieties of gladiolus for various morphological parameters (Table 1), thereby indicating tremendous potential for improvement through selection. The perusal of data presented in Table 2 revealed that varieties showed variation for days taken to sprouting of corms. Variety Praha took minimum days to sprouting (5.17 days) which was at par with Essley Blue (6.00 days). However, Nova Lux took maximum days to sprouting (10.40 days). Maximum number of leaves was recorded in variety Big Time Supreme (9.84) which was at par with Praha (9.80) followed by Nova Lux (9.67), Flirt (9.07) and Essley Blue (9.00). The least number of leaves per plant was found in variety Berlew (7.42). The data pertaining to the plant height varied significantly and maximum plant height was observed in variety Shabnam (105.47 cm) which was statistically at par with Suchitra (100.63 cm). However, minimum plant height was observed in variety Yellow Stone (74.93 cm). The above result indicates that different varieties have different dormancy periods and it may be

controlled by genetic factors. The variation in number of leaves per plant and plant height in different varieties may be due to genetic and environmental factors. Similar results were also reported by Sidhu and Arora (2000) and Basavaraddy (2004), Singh *et al.*, (2017); Kumar *et al.* (2018); Kumawat *et al.* (2018); Swaroop *et al.* (2018); Bhaskar *et al.* (2019) and Nalage *et al.* (2019) in gladiolus.

Different varieties of gladiolus exhibited pronounced variation for flowering characters viz., days taken to spike emergence, days to opening of first floret, floret size, number of florets per spike, spike length, rachis length etc. (Table 2). The variety Gunjan took minimum days to spike emergence (66.67 days) which was statistically at par with variety Creamy Green (72.67 days), Berlew (72.73 days), Yellow Stone (73.33 days), Viola (73.73 days) and Shabnam (74.32 days). Big Time Supreme took maximum days to spike emergence (86.69 days). In case of opening of first floret, variety Gunjan was earliest (86.21 days) whereas Nova Lux took maximum days to opening of first floret (101.80 days). The variation in days taken to spike emergence and days taken to opening of first floret might be attributed to genetic makeup of the cultivars, which is governed by the genotypic constituent of the plant and it may also be influenced with growing environment of the plants. Similar results for spike emergence and days taken to opening of first floret were also reported by Kumar and Yadav (2005); Rani *et al.* (2007); Bhaskar *et al.* (2019); Safeena and Thangam (2019) and Nalage *et al.* (2019) in gladiolus.

Maximum floret size was recorded in variety Essley Blue (9.82 cm), and it was at par with Big Time Supreme (9.77cm), Viola (9.45 cm), American Beauty Pink (9.43cm), Praha (9.09 cm), Gunjan (8.89 cm) and Nova Lux (8.79 cm). Minimum Floret size was found in variety Suchitra (7.05 cm). Number of florets opened at one time was recorded

maximum in Nova Lux (8.47) and it was statistically at par with Viola (8.13). However, number of florets opened at one time was recorded minimum in Shabnam (5.95). Perusal of data for duration of flowering revealed that variety Shabnam showed maximum duration of flowering (17.98 days). However, variety Flirt showed minimum flowering duration (13.40 days). Number of florets per spike was recorded maximum in variety Shabnam (14.22) and variety Berlew exhibited minimum number of florets per spike (8.13).

Spike characters are important with regard to cut flower production in gladiolus especially when we consider quality (Table 3). The range of the spike length varied from 67.07 cm to 89.93 cm. Maximum spike length was recorded in variety Essley Blue (89.93 cm) and it was at par with Shabnam (89.44 cm), Suchitra (83.09 cm), Praha (81.56 cm), American Beauty Pink (77.73 cm) and Nova Lux (77.67 cm). The variety Flirt exhibited minimum spike length of 67.07 cm. Rachis length was found maximum in variety Essley Blue (55.64 cm) and it was at par with Shabnam (51.53 cm), Suchitra (48.96 cm), Big Time Supreme (48.05 cm), Nova Lux (47.63 cm) and Viola (47.22 cm). The variation in floral characters might be due to hereditary traits of the varieties, which is governed by genetic makeup of the plants. Similar results on these flowering attributes have been reported by Rani and Singh (2005); Mahawer *et al.* (2013); Chourasia *et al.* (2015); Kujur *et al.* (2016); Sarkar *et al.* (2016); Singh *et al.* (2018) and Nagar *et al.* (2017) in gladiolus.

The perusal of data presented in Table 3 reveal that variety Suchitra recorded maximum vase life of 17.33 days. This variation in vase-life might be due to differential accumulation of carbohydrates, sensitivity of varieties to ethylene and genetic makeup of the plant. The results are in line with the findings of Momin *et al.* (2015);

Chaudhary and Dhatt (2017); Bhaskar *et al.* (2019) and Nalage *et al.* (2019) in gladiolus. The water uptake varied from 17.33 ml to 46.67 ml. The variety Creamy Green showed maximum water uptake (46.67 ml). However, variety Shabnam had minimum water uptake (17.33 ml) and it was at par with Yellow Stone (29.33 ml).

Corm diameter and corm weight are important parameters for producing quality spikes along with more number of florets of larger size. An inquisition of data presented in Table 3 reveals that maximum number of corms per plant was found in variety Viola (2.27) followed by Creamy Green (2.06). The variety Yellow Stone, Gunjan and Berlew recorded minimum number of corm per plant (1.00). Maximum corm diameter was found in variety Praha (6.77 cm) and variety Viola exhibited minimum corm diameter (5.60 cm). Corm weight ranged from 39.97 g to 100.00 g. The maximum corm weight was recorded in the variety Praha (100.00 g) and it was statistically at par with Nova Lux (86.83 g). The minimum corm weight was exhibited by variety Gunjan (39.97 g) and it was at par with Mayur (46.33 g). In gladiolus, corm production directly depends on number of shoots produced per mother corm as reported by Ramachandrudu and Thangam (2008). The

production of daughter corms of more weight may be attributed to the good vegetative growth of plants in the initial stages, which supplies higher amounts of photosynthates for storage in the corms which are also the storage organs (Kumar, 2017). Similar variation for number of corms per plant, corm diameter and corm weight in gladiolus was also observed by Das *et al.* (2014); Kujur *et al.* (2016); Kaur and Bajpay (2019); Nalage *et al.* (2019) and Safeena and Thangam (2019) in gladiolus.

Significant differences were noticed among the varieties with respect to number of cormels per plant. The maximum number of cormels per plant was recorded in variety Essley Blue (41.97). However, minimum number of cormels per plant was found in variety Gunjan (4.47). Maximum weight of cormels per plant was found in variety Essley Blue (13.42 g) and it was at par with variety Berlew (10.77 g). Minimum weight of cormels per plant was recorded in variety Shabnam (2.01 g). Number, size and weight of cormels produced per plant may vary from variety to variety due to variation in their genetic makeup. Results are in confirmation with the findings of Kumar *et al.* (2018); Kaur and Bajpay (2019); Nalage *et al.* (2019) Safeena and Thangam (2019) in gladiolus.

Table 1 Analysis of variance of 19 traits of fifteen genotypes of gladiolus

Source of variation	df	Mean sum of square									
		1	2	3	4	5	6	7	8	9	10
Replication	2	1.83	107.44	16.44	1.78	2.41	5.53	39.07	0.49	6.36	214.58
Genotypes	14	6.80	78.10	72.80	2.96	1.41	5.90	9.47	0.13	1.83	271.59
Error	28	2.55	26.94	8.53	0.50	1.07	2.47	2.71	0.07	0.34	68.24

Source of variation	df	Mean sum of square								
		11	12	13	14	15	16	17	18	19
Replication	2	343.14	37.19	4.83	8.87	0.067	0.87	5.58	1.42	15.21
Genotypes	14	242.49	99.01	8.64	168.39	0.40	0.35	706.06	340.88	32.16
Error	28	81.37	44.31	1.54	48.98	0.01	0.10	66.92	68.07	9.06

Where 1,2,3 stands for.....

1	Days taken to sprouting of corms	8	Number of spikes per plant	15	Number of corms per plant
2	Days taken to spike emergence	9	Number of leaves per plant	16	Corm diameter (cm)

3	Days taken to opening of first floret	10	Plant height(cm)	17	Corm weight (g)
4	Floret size(cm)	11	Spike length (cm)	18	Number of cormels per plant
5	Number of florets opened at one time	12	Rachis length (cm)	19	Weight of cormels per plant (g)
6	Duration of flowering (Days)	13	Vase life (Days)		
7	Number of florets per spike	14	Water uptake (ml)		

Table 2: Performance of gladiolus varieties w.r.t. to vegetative and flowering attributes.

Variety	Days taken to sprouting of corms	Number of leaves per plant	Plant height (cm)	Days taken to spike emergence	Days taken to opening of first floret	Floret size (cm)	Number of florets opened at one time	Duration of flowering (days)	Number of florets per spike
Creamy Green	7.07	8.00	77.12	72.67	91.15	8.10	6.47	16.07	10.80
Flirt	7.07	9.07	79.60	77.00	92.93	7.10	6.19	13.40	9.93
Berlew	6.93	7.42	78.21	72.73	88.66	7.94	7.13	14.44	8.13
Mayur	8.00	8.00	86.23	78.60	94.73	7.29	7.53	16.47	12.33
Viola	7.67	8.18	93.27	73.73	90.29	9.45	8.13	16.45	11.11
Gunjan	8.27	8.48	82.27	66.67	86.21	8.89	7.58	16.83	10.82
Suchitra	8.67	8.33	100.63	84.58	101.25	7.05	6.67	16.00	13.97
Yellow Stone	8.00	8.20	74.93	73.33	88.33	8.21	7.05	16.92	13.13
Shabnam	9.40	8.12	105.47	74.32	91.46	8.73	5.95	17.98	14.22
Snow Princess	10.00	7.50	91.36	76.58	90.28	7.08	7.25	15.25	12.08
Essley Blue	6.00	9.00	98.87	77.53	93.93	9.82	7.07	13.47	11.13
Big Time Supreme	9.80	9.84	97.04	86.69	101.78	9.77	7.78	14.35	10.55
American Beauty Pink	6.87	8.90	90.25	75.89	93.33	9.43	7.13	14.59	9.72
Praha	5.17	9.80	97.89	82.53	96.07	9.09	6.80	13.67	12.47
Nova Lux	10.40	9.67	90.67	78.27	101.80	8.79	8.47	15.20	14.20
C.D. (p=0.05)	2.67	0.97	13.82	8.68	4.89	1.18	1.72	2.63	2.75

Table 3: Performance of gladiolus varieties w.r.t. to flowering, post harvest and corm parameters

Variety	Number of spikes per plant	Spike length (cm)	Rachis length (cm)	Vase life (days)	Water uptake (ml)	Number of corms per plant	Corm diameter (cm)	Corm weight (g)	Number of cormels per plant	Weight of cormels per plant (g)
Creamy Green	1.27	64.22	38.24	15.92	46.67	2.06	6.65	80.04	19.97	6.57
Flirt	1.67	67.07	33.89	11.48	30.00	1.27	6.46	76.94	9.67	4.53
Berlew	1.53	62.95	37.53	12.33	40.00	1.00	5.99	56.64	19.93	10.77
Mayur	1.20	73.13	40.41	15.63	33.33	1.13	6.06	46.33	7.63	2.56
Viola	1.40	77.73	47.22	15.33	37.33	2.27	5.60	57.41	19.83	4.57
Gunjan	1.07	67.24	41.22	17.00	34.33	1.00	5.95	39.97	4.47	3.24
Suchitra	1.00	83.09	48.96	17.33	38.33	1.27	6.38	73.32	6.27	2.45
Yellow Stone	1.27	62.20	42.00	14.83	29.33	1.00	6.61	64.41	9.73	4.42
Shabnam	1.27	89.44	51.53	15.58	17.33	1.27	5.75	56.37	4.80	2.01
Snow Princess	1.08	71.48	44.84	16.83	45.00	1.20	6.03	67.17	4.54	3.79
Essley Blue	1.47	89.93	55.64	15.75	33.00	1.27	6.42	62.09	41.97	13.42
Big Time Supreme	1.07	71.93	48.05	16.80	36.00	1.40	6.17	67.49	25.67	7.99
American Beauty Pink	1.31	77.73	45.50	14.15	36.00	1.20	6.13	64.84	25.03	8.32
Praha	1.47	81.56	46.41	16.43	45.00	1.40	6.77	100.00	14.60	5.12
Nova Lux	1.00	77.67	47.63	16.78	42.33	1.20	6.47	86.83	23.75	7.65
CD (p=0.05)	0.45	15.09	11.13	2.08	11.71	0.19	0.54	13.68	13.80	5.04

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Received : January 2026; Revised : February 2026; Accepted : March 2026



PRECISION APPLICATION OF NITROGEN BY ROOT DIPPING APPLICATION AS STARTER SOLUTION IN CHILLI (*CAPSICUM ANNUUM* L.)

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Abstract

To meet the nutritional demand of the vegetable crops, it is essential to supply nutrients throughout their growth period. Supply of nutrients at initial growth stages of plant leads to improved root development thus ultimately enhances nutrient uptake. Application of fertilisers near the plant root zone had been found more beneficial in liquid form as compared to solid applications. Therefore, an experiment was conducted that comprised one control N₁ (water dip only) and three concentrations of urea solution viz., N₂ (1% urea solution), N₃ (2% urea solution) and N₄ (3% urea solution) applied as root dip to chilli hybrid i.e., G₁ (Arka Gagan) and variety namely G₂ (Kashi Anmol). All the treatments were laid out in Factorial Randomized Block Design (FRBD) with three replications. The data observed for all the parameters was found significantly affected with the use of varying concentration of urea solution. All the growth and yield parameters were statistically improved with increased concentration of urea solution from 0 to 3 per cent, while the least days to first flowering (87.50) and days to 50% flowering (94.83) and days to first harvest (112.83) were observed under control treatment. Performance of the hybrid (Arka Gagan-962.1 g/plant) and variety (Kashi Anmol-879.7 g/plant) in terms of yield was improved by 24 % and 19.4% respectively, with the application of 3% urea solution as root dipping as compared to control treatment (water dip).

Keywords: Root priming, Liquid fertilizers, Nitrogen, Starter solution, Urea

INTRODUCTION

Chilli or Hot pepper (*Capsicum annuum* L.) is the third most important vegetable and spice crop of family Solanaceae, following tomato and potato (29). It is a warm season crop that requires humid temperature to grow and a dry climate to mature. It is semi-perennial in nature, but commonly grown as an annual crop. Chilli exhibits several important minerals and bioactive

compounds that have antioxidant, antibacterial, antiviral, anti-inflammatory and anticancer properties (24). Fresh green chillies have more vitamin C (242 mg/100 gm) than citrus fruits (70 mg/100 gm) (27). Besides adding flavour, chilli also have important nutraceutical value (33). Capsaicin is currently used for the treatment of diabetic neuropathy, osteoarthritis, post-herpetic neuralgia, and psoriasis (22).

Being a largest consumer, producer and exporter of chilli, India produced 4.7 million tonnes from an area of 4.3 lakh ha with productivity of 8.4 tonne/ha (28). The productivity of crops depends upon various biotic and abiotic factors among which fertilization is considered as most important (15), (14), (26). Nitrogen is an important nutrient that affects growth and photosynthetic rates, thus productivity (9), (31), (45). Only a small amount (10% to 40%) of nitrogenous fertilizer is taken up by plants (8) most of it is lost due to hydrolysis, leaching and microbial immobilisation or degradation (17), (19). Therefore, the precision of amount, duration and method of nitrogenous fertilizer application is of utmost importance.

Application of fertilizers in liquid form found to be more beneficial if placed near the plant root zone at early growth stages of plant (12), (32). It makes the nutrients more readily available thus significantly increases the nutrient uptake by plant roots. The increased efficiency with liquid applications decreases the fertilizer requirement up to 50% less than conventional solid applications (25). Providing nutrition at early stage in liquid form can also help to reduce the transplanting shock. Applications of phosphate fertilizer as starter solution at three days after transplanting observed the higher survival rate of tomato seedlings (13). Urea containing starter solutions have also been found to improve plant growth of different vegetables like chilli, cabbage, cauliflower and tomato (12), (32), (39). In this context, the research was aimed at finding the correct dose of urea solution application as starter solution for quality production of chilli with higher productivity.

MATERIALS AND METHODS

Experimental site

The present investigation entitled “Precision application of nitrogen by root dipping application as starter solution in chilli

(*Capsicum annuum* L.)” was conducted at experimental Vegetable Research Farm, P.G. Department of Agriculture, Khalsa College, Amritsar during 2022-2023.

Planting materials

The seeds of hybrid Arka Gagan were procured from IIHR, Bangalore, Karnataka and variety Kashi Anmol from IIVR, Varanasi, Uttar Pradesh, India. The seeds were sown in plug trays filled with a soilless mixture of cocopeat, perlite and vermiculite (3:1:1) during the second fortnight of October in the year, 2022.

Experimental methodology

The urea solutions of different concentration i.e., 1%, 2 % and 3 % were prepared by dissolving the required amount of urea fertilizer (46% N) per litre of water. The control treatment was kept as water dip only (0% urea solution). Before transplanting the seedlings at the age of thirty-five days, the seedlings were uprooted from the plug trays and growing medium was removed by shaking or slightly hitting to ground. The seedling roots of both Arka Gagan and Kashi Anmol were dipped into urea containing solutions. The treated seedlings were planted on one side of the ridges at a spacing of 75 cm × 45 cm. The field was slightly irrigated just after transplanting. The experiment consisted of eight treatments replicated thrice that were laid out in factorial randomised block design. There were fifteen plants transplanted in each treatment per replication. The whole experiment was performed under low tunnel technology to save the seedlings from frost till the middle of February. All the package and practices were followed for raising a good crop as per given in the book prepared by Punjab Agricultural University, Ludhiana (3).

Observations recorded

The data for different plant parameters were determined from six randomly selected

plants in each replication per treatment and the average was calculated. The data was collected as follows;

Growth parameters

The plant height (cm) at fifteen days interval was measured from base to tip of the last leaf with the help of measuring tape. The number of primary and secondary branches in plant was counted at final harvest and their sum was used as number of total branches per plant. The length of longest root was measured with the help of measuring scale from stem base to the end point of root after final harvest by uprooting the plants.

Flowering parameters

The number of days taken from the date of transplanting to the appearance of first flower was counted and expressed as number of days to first flowering. The number of days taken from transplanting to the opening of flowers on 50% plants in each treatment per replication were noted down and expressed as days to 50% flowering. The number of days taken from transplanting to harvest of at least one ripe fruit in each treatment per replication were counted and expressed as days to first harvest.

Fruit and yield parameters

The data for fruit parameters was taken from randomly selected six fruits in each treatment per replication in the middle of harvesting and the average was computed. The fruit length was measured from the apex to base of fruit leaving peduncle. Average fruit weight (g) was worked out by weighing randomly selected five fruits in each treatment per replication and average was worked out. The total number of fruits harvested in each picking was counted, adding the number over all pickings and average of six plants was worked out to express number of fruits per plant. Fruit yield per plant was

calculated by adding marketable yield of randomly selected six plants in each picking and their averaged was calculated. The total yield per hectare (q/ha) was calculated by multiplying the yield per plant with the number of plants in a hectare.

Statistical analysis

The data pertaining to the present investigation were statistically analysed at the 5% level of significance using OPSTAT software (36).

RESULTS

Growth parameters

Plant height

Significant variation was recorded in plant height of chilli due to different concentrations of urea solution at all (15, 30, 45, 60 and 90) days after transplanting (DAT) (Table 1). The plant growth is represented with their respective treatments in the Fig. 1. At 15 DAT, the tallest plant height (14.13 cm) was recorded from N_4 (3% urea solution) followed by 12.03 cm under N_3 (2% urea solution), which was statistically at par with 11.10 cm under N_2 (1% urea solution) treatment. While, the shortest plant height (9.48 cm) was recorded from control, where no urea solution as root dipping was applied. Similarly at 30 DAT, the tallest plant height (18.59 cm) was recorded in N_4 (3% urea solution) followed by N_3 (2% urea solution) and N_2 (1% urea solution) 17.38 cm and 15.02 cm, respectively. The shortest plant height of 12.35 cm was recorded from N_1 (0% urea solution) treatment at 30DAT. Same trends of growth in plant height was observed as tallest plant height of 26.48, 30.75 and 34.11 cm under N_4 (3% urea solution) treatment at 45, 60 and 90 DAT, respectively. The shortest plant height of 20.18, 24.21 and 29.00 cm at 45, 60 and 90 DAT, respectively was observed under N_1 (0% urea solution) treatment.

Among genotypes, Arka Gagan achieved maximum height of 15.25 cm, 20.54 cm, 28.82 cm, 33.64 cm and 37.85 cm than Kashi Anmol genotype as 8.11 cm, 11.12 cm, 17.76 cm, 21.27 cm and 25.04 cm at all 15, 30, 45, 60 and 90 DAT respectively. Combined effect of different concentrations of urea solution and the genotypes showed significant differences for plant height only at 15 and 30 DAT. The difference in plant height as combined effect of urea solution and genotypes at 45, 60 and 90 DAT was found statistically non-significant. However, at 15 DAT the tallest plant (18.73 cm) was recorded from G_1N_4 (Arka Gagan and 3% urea solution) the shortest plant height (6.93 cm) was recorded from G_2N_2 (Kashi Anmol and 1% urea solution). At 30 DAT the tallest plant (24.58 cm) was recorded from G_1N_4 (Arka Gagan and 3% urea solution) which was statistically at par with G_1N_3 (Arka Gagan and 2% urea solution) (22.87 cm) while the shortest plant was recorded from G_2N_1 (Kashi Anmol and 0% urea solution) (9.50 cm).

Number of branches per plant

The highest number of branches (30.07) was recorded under N_4 (3% urea solution) followed by N_2 with 26.35 and N_3 with 25.46 while the lowest number of branches (25.00) was recorded under N_1 (1% urea solution). It was evident from the results, that number of branches per plant increased with the application of urea solution at higher concentration i.e., 3% urea solution. While, the concentration lower than 3% urea solution did not influenced the number of branches per plant. Genotype Arka Gagan observed statistically higher number of branches (27.74) than the genotype Kashi Anmol (25.70). Similarly, the significant interaction effect was observed where the highest number of branches (31.67) per plant was recorded from G_1N_4 (Arka Gagan and 3% urea solution)

followed by 28.47 under G_2N_4 (Kashi Anmol and 3% urea solution).

Root length (cm)

The longest root (22.58 cm) was measured from the treatment N_4 (3% urea solution) followed by 17.91 cm under N_3 (2% urea solution) which was statistically at par with 16.36 cm under N_2 (1% urea solution), while the shortest root length (13.10 cm) was measured from N_1 (0% urea solution) that is presented in table 1. The length of plant roots with their respective treatments is presented in Fig. 2. Among genotypes, the longest (19.12 cm) root length was measured from the genotype Arka Gagan than in the genotype Kashi Anmol (15.85 cm). The interaction effect of urea solution and genotypes showed significant variation where the longest root (26.66 cm) was measured under G_1N_4 (Arka Gagan and 3% urea solution) treatment combination followed by 19.50 cm under G_1N_3 (Arka Gagan and 2% urea solution), while the shortest root length (13.03 cm) was measured in the treatment combination G_2N_1 (Kashi Anmol and 0% urea solution) statistically at par with 13.16 cm under G_1N_1 (Arka Gagan and 0% urea solution).

Flowering parameters

Data presented in the Table no. 2 revealed that the minimum days to first flowering (87.50), 50% flowering (94.83) and first harvesting (112.83) observed under control whereas maximum days to first flowering (92.50), 50% flowering (100.33) and first harvesting (117.83) was recorded under 3% starter solution. Among genotypes, Arka Gagan took the minimum days to first flowering (95.50), 50% flowering (99.41) and first harvesting (119.16) as compared to the genotype Kashi Anmol. Interaction effect of starter solution and genotypes observed non-significant for the days to first flowering, 50% flowering and for the first harvesting.

Fruit and yield parameters

All the fruit parameters were statistically influenced with use of different concentrations of urea solution (Table 3). The maximum average fruit length (7.89 cm), fruit diameter (9.50 mm) and fruit weight (2.87 g), number of fruits per plant (382.57), yield per plant (1019.74 g) and yield per hectare (302.14 q/ha) were measured under the 3% starter solution treatment and the minimum average fruit length (7.06 cm), fruit diameter (7.10 mm) and fruit weight (2.64 g), number of fruits per plant (337.72), yield per plant (836.96 g) and yield per hectare (247.98 q/ha) were measured under control treatment (0% urea solution). Among different chilli genotypes, significantly maximum number of fruits per plant (465.14), yield per plant (962.13 g) and yield per hectare (285.07 q/ha) was found in genotype Arka Gagan whereas maximum fruit diameter (8.60 mm) and fruit weight (3.44 g) was observed in Kashi Anmol. While, the variation in fruit length between different chilli genotypes was observed non-significant. The interaction effect of starter solution and genotypes, the maximum fruit diameter (10.0 mm) and fruit weight (3.62 g) was observed under 3% urea solution applied to Arka Komal (G_2N_4). While, the maximum number of fruits per plant (505.84) were observed under the treatment combination of Arka Gagan at 3% urea solution (G_1N_4). Whereas, the interaction effect of genotypes and urea solution was found non-significant for yield per plant (g) and yield (q/ha) in chilli.

Table 1 Effect of root dipping application as starter solution on growth parameters of chilli

		Plant height (cm) at different days					Number of branches per plant	Root length (cm)
Treatments		15 Days	30 Days	45 Days	60 Days	90 Days		
Genotypes	G ₁ (Arka Gagan)	15.25 ^a	20.54 ^a	28.82 ^a	33.64 ^a	37.85 ^a	27.74 ^a	19.12 ^a
	G ₂ (Kashi Anmol)	8.11 ^b	11.12 ^b	17.76 ^b	21.27 ^b	25.04 ^b	25.70 ^b	15.85 ^b
	CD (p= 0.05)	0.93	1.24	2.06	2.27	2.22	0.67	1.34
	SE(m)	0.30	0.40	0.67	0.74	0.72	0.21	0.43
Urea solution	N ₁ (0% urea solution)	9.48 ^c	12.35 ^d	20.18 ^b	24.21 ^b	29.00 ^b	25.00 ^c	13.10 ^c
	N ₂ (1% urea solution)	11.10 ^b	15.02 ^c	22.57 ^b	27.38 ^b	30.80 ^b	26.35 ^b	16.36 ^b
	N ₃ (2% urea solution)	12.03 ^b	17.38 ^b	23.95 ^b	27.48 ^b	31.86 ^b	25.46 ^b	17.91 ^b
	N ₄ (3% urea solution)	14.13 ^a	18.59 ^a	26.48 ^a	30.75 ^a	34.11 ^a	30.07 ^a	22.58 ^a
	CD (p= 0.05)	1.32	1.76	2.91	3.22	3.15	0.95	1.89
	SE(m)	0.43	0.57	0.95	1.05	1.02	0.31	0.62
Interaction	Genotype × Urea solution							
	G ₁ N ₁	11.30 ^c	15.2 ^c	23.93	28.86	33.23	25.80 ^c	13.16 ^{ef}
	G ₁ N ₂	15.26 ^{bc}	19.54 ^b	27.91	32.97	37.36	28.21 ^b	17.16 ^{bcd}
	G ₁ N ₃	15.73 ^b	22.87 ^{ab}	29.86	34.56	39.10	25.27 ^c	19.50 ^b
	G ₁ N ₄	18.73 ^a	24.58 ^a	33.60	38.16	41.70	31.67 ^a	26.66 ^a
	G ₂ N ₁	7.66 ^e	9.50 ^g	16.43	19.56	24.76	24.20 ^e	13.03 ^f
	G ₂ N ₂	6.93 ^f	10.50 ^f	17.23	21.80	24.23	24.50 ^{de}	15.56 ^{de}
	G ₂ N ₃	8.33 ^{de}	11.90 ^e	18.03	20.40	24.63	25.46 ^{de}	16.33 ^{cd}
	G ₂ N ₄	9.53 ^d	12.60 ^d	19.36	23.33	26.53	28.47 ^b	18.50 ^{bc}
	CD (p= 0.05)	1.87	2.49	N/S	N/S	N/S	1.34	2.68
	SE(m)	0.61	0.81	1.34	1.48	1.45	0.43	0.87

Table 2 Effect of root dipping application of starter solution on flowering parameters of chilli
Treatments **Number of days to first flowering** **Number of days to 50% flowering** **Number of days to first harvesting**

	Treatments	Number of days to first flowering	Number of days to 50% flowering	Number of days to first harvesting
Genotypes	G ₁ (Arka Gagan)	95.50 ^a	99.41 ^a	119.16 ^a
	G ₂ (Kashi Anmol)	84.58 ^b	95.58 ^b	111.50 ^b
	CD (p= 0.05)	0.96	0.92	0.86
	SE(m)	0.31	0.30	0.28
Urea solution	N ₁ (0% urea solution)	87.50 ^d	94.83 ^d	112.83 ^d
	N ₂ (1% urea solution)	89.50 ^c	96.50 ^c	114.66 ^c
	N ₃ (2% urea solution)	90.66 ^b	98.33 ^b	116.00 ^b
	N ₄ (3% urea solution)	92.50 ^a	100.33 ^a	117.83 ^a
	CD (p= 0.05)	1.36	1.31	1.21
	SE(m)	0.44	0.42	0.39
Interaction	Genotype × Urea solution			
	G ₁ N ₁	93.00	96.33	116.66
	G ₁ N ₂	95.00	98.00	118.33
	G ₁ N ₃	96.00	100.66	119.66
	G ₁ N ₄	98.00	102.66	122.00
	G ₂ N ₁	82.00	93.33	109.00
	G ₂ N ₂	84.00	95.00	111.00
	G ₂ N ₃	85.33	96.00	112.33
	G ₂ N ₄	87.00	98.00	113.66
	CD (p= 0.05)	N/S	N/S	N/S
	SE(m)	0.62	0.60	0.56

Table 3 Effect of root dipping application of starter solution on fruit and yield parameters of chilli

	Treatments	Fruit length (cm)	Fruit diameter (mm)	Fruit weight (g)	Number of fruits per plant	Yield per plant (g)	Yield per hectare (q/ha)
Genotypes	G ₁ (Arka Gagan)	7.49	8.33 ^b	2.06 ^b	465.14 ^a	962.13 ^a	285.07 ^a
	G ₂ (Kashi Anmol)	7.40	8.60 ^a	3.44 ^a	253.57 ^b	879.70 ^b	260.64 ^b
	CD (p= 0.05)	N/S	0.19	0.06	10.79	22.31	6.61
	SE(m)	0.06	0.06	0.02	3.52	7.28	2.15
Urea solution	N ₁ (0% urea solution)	7.06 ^c	7.1 ^d	2.64 ^d	337.72 ^b	836.96 ^d	247.98 ^d
	N ₂ (1% urea solution)	7.29 ^{bc}	8.35 ^c	2.74 ^c	348.30 ^b	889.18 ^c	263.45 ^c
	N ₃ (2% urea solution)	7.53 ^b	8.93 ^b	2.76 ^b	368.84 ^{ab}	937.78 ^b	277.85 ^b
	N ₄ (3% urea solution)	7.89 ^a	9.5 ^a	2.87 ^a	382.57 ^a	1019.74 ^a	302.14 ^a
	CD (p= 0.05)	0.27	0.27	0.08	15.27	31.55	9.34
	SE(m)	0.09	0.09	0.02	4.98	10.30	3.05
Interaction	Genotype × Urea solution						
	G ₁ N ₁	7.04	7.9 ^f	2.02 ^d	428.53 ^d	868.48	257.32
	G ₁ N ₂	7.39	8.1 ^{ef}	2.07 ^d	445.00 ^c	920.97	272.87
	G ₁ N ₃	7.68	8.3 ^{de}	2.04 ^d	481.19 ^b	981.57	290.83
	G ₁ N ₄	7.84	8.9 ^c	2.13 ^d	505.84 ^a	1077.49	319.25
	G ₂ N ₁	7.08	6.3 ^g	3.26 ^c	246.90 ^e	805.45	238.64
	G ₂ N ₂	7.19	8.6 ^{cd}	3.41 ^b	251.59 ^e	857.39	254.03
	G ₂ N ₃	7.38	9.5 ^b	3.49 ^b	256.49 ^e	894.00	264.88
	G ₂ N ₄	7.94	10.0 ^a	3.62 ^a	259.31 ^e	961.99	285.03
	CD (p= 0.05)	N/S	0.38	0.12	21.59	N/S	N/S
	SE(m)	0.12	0.12	0.04	7.05	14.56	4.31

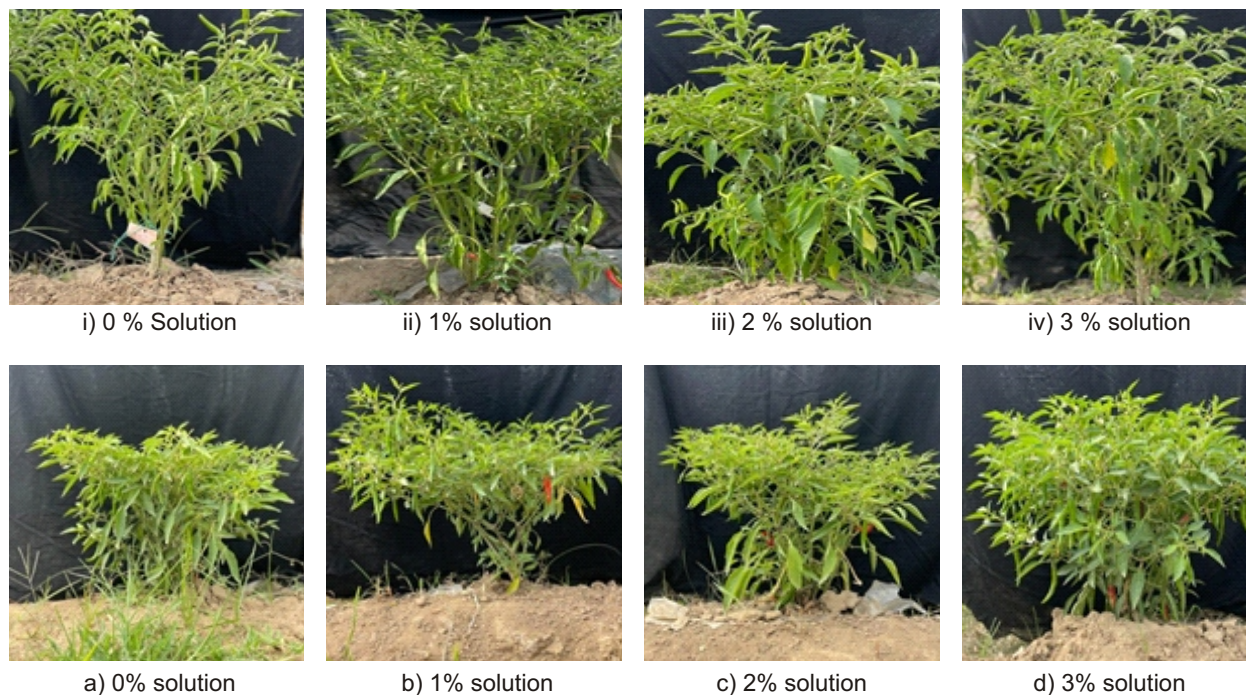


Fig 1. Effect of root dipping on plant growth of chilli where fig i, ii, iii and iv represents plant growth of hybrid Arka Gagan and fig a, b,c and d represents the plant growth of variety Kashi Anmol

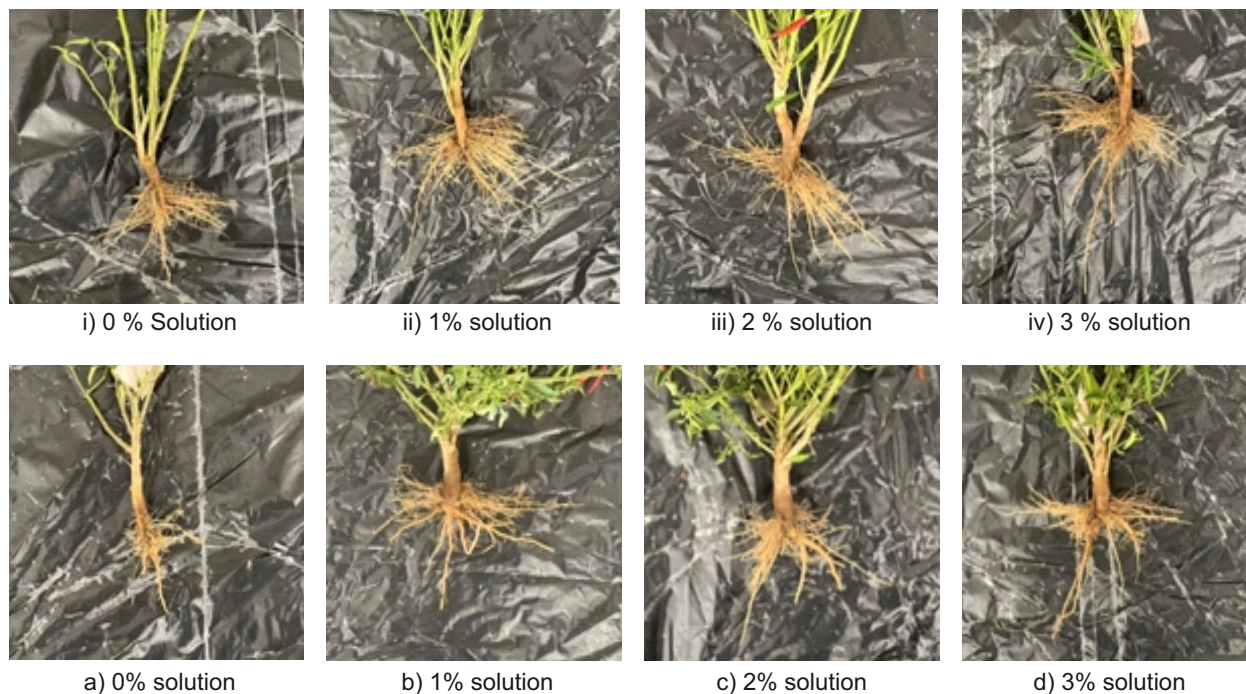


Fig 2. Effect of root dipping application of starter solution on root growth of chilli where fig i, ii, iii and iv represents root growth of hybrid Arka Gagan and fig a, b,c and d represents the fruits of variety Kashi Anmol

DISCUSSION

All the growth parameters like plant height, total number of branches per plant and root length were significantly improved with the increasing concentration of urea as starter solution. Moreover, the maximum plant height at all days, total number of branches (27.74) and root length (19.12 cm) was measured under 3% starter solution whereas, the minimum plant height, total number of branches per plant (25.00) and root length (25.00cm) was recorded under control. The increased plant height with increased concentration of nitrogen was also observed by (38) and (23). The application of nitrogen enhances the vegetative growth that ultimately improved the number of branches per plant (5). Root length increased with increasing levels of nitrogen was also observed by (41) in chilli and in cabbage by (30). At initial growth period up to 30 DAT, the interaction of cultivars with higher concentration of urea solution (3%) application produced maximum plant height. The results are in line with study conducted by (10) who also observed the significantly highest plant height (cm) from interaction of cultivars and nitrogen applications as compare to control treatment. While, at later growth stage from 45 to 90 DAT, the interaction effect of chilli genotypes and concentration of urea solution was not significant. Similar results of non-significant interaction between cultivars and nitrogen level was observed by (43).

The delay in flowering with increased application of nitrogen fertilization may be due to the diversion of photosynthates for vegetative growth instead of reproductive phase (18). Similar results were reported in capsicum by (40) and in potato by (34). The delayed flowering leads to increased days in first fruit harvest that was also observed by (42) and (7) in chilli. The different responses of the genotypes for the flowering time might be related to genetic variation (43). Similar

results of late first harvest in Calabrese type broccoli (55.81 days to) than the sprouting green (81.19 days) type broccoli were observed under the different level of nitrogen application by (16).

The availability of important nutrient supplement i.e. nitrogen at early growth stage may helped to boost fruit length (cm), fruit diameter (mm) and fruit weight (g) that was also reported in studies conducted by (41), (32), (20), (1). Moreover, the nitrogen improves vegetative growth that produce more number of branches thus more number of fruits (41). The similar results of increased number of fruits with increased nitrogen applications were reported by (32). The response of additional nitrogen may be due to the fact that plant produce more leaves would have increased photosynthetic and hence better performance in terms of yield (35). (37) and (2) founds similar result of significantly increase in yield per plant with the increased nitrogen in broccoli and pepper. The higher average fruit weight and higher number of fruits per plant could be another reason for high yield (21), (44), (6) and (11).

CONCLUSION

Application of urea solution as root dipping improved the growth and yield parameters of chilli. All the parameters were found to be improved with increased concentration of urea solution except for flowering that was delayed. Both the hybrid Arka Gagan and variety Arka Komal performed positively to applied urea solution as root dipping that recorded higher yield of 24 % and 19.4% per plant respectively compared to control (water dip).

ACKNOWLEDGEMENT

Authors are thankful to the authorities of Khalsa College, Amritsar for providing the required resources and support to conduct this research experiment. Authors are also thankful to the PG Department of Khalsa

College for providing all facilities to conduct the experiment. The first author is the student of Gagandeep Singh, for his M.Sc. thesis submitted to Guru Nanak Dev University, Amritsar.

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ISOLATION, SCREENING, AND MORPHOLOGICAL CHARACTERIZATION OF LOW-DENSITY POLYETHYLENE (LDPE)-DEGRADING FUNGI FROM THE WASTE YARDS

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Abstract

Low-density polyethylene (LDPE) is one of the most popular and least biodegradable synthetic polymers, and polymer manufacturing plants generate plastic waste that needs more serious study as a bioprospecting location for LDPE degrading fungi. In total, seven locations (which include both polyethen factories' waste dump sites and reference zones polluted by polymers) were sampled for indigenous soil fungi by using serial dilution and enrichment methods. The results of clear-zone and LDPE film loss tests showed that the most abundant isolate from the polymers factory dump had unbranched and erect septate conidiophores with globose vesicles with biseriaphialides and chains of dark brown/black coarsely roughened conidia typical for the genus *Aspergillus*. In the preliminary quantitative screening, this isolate (F1) showed $23.79 \pm 1.52\%$ LDPE film loss after the incubation period of 30 days which corresponds to the literature data for LDPE degrading members of the family *Aspergillus* recorded elsewhere. Three more isolates (F2-F4) were also subject to the same clear-zone and weight-loss tests showing further evolution of the results.

Keywords: *low-density polyethylene; LDPE biodegradation; plastic-degrading fungi; Aspergillus niger; waste-yard soil; bioremediation; plastic pollution*

INTRODUCTION

Plastic production has risen significantly, with the figure of virgin plastic reaching 8300 million metric tons in 2015, most of which has been contaminated with waste and is not treated (Geyer *et al.*, 2017). Polyethylene is a popular option for packaging, as well as for making bags, industrial wrap, and other similar items due to its low price, flexibility, and moisture-resisting properties. It can also be noted that the same properties that make it

chemically stable — its long hydrophobic structure — also make it possible for LDPE to resist environmental degradation. Landfilling and burning plastic waste are seen as insufficient methods for dealing with waste: landfilling takes up space, creates leachate, while incineration produces gases (Sen & Raut, 2015). Therefore, biodestruction using fungi and their enzymes is the solution against plastic pollution. Fungus is a potential option due to its capability of producing

oxidizing and hydrolyzing enzymes that attack the structure of the polyethylene polymer. Over the last twenty years numerous researchers have studied their activity and found many fungi that can degrade LDPE.

Soil near and in polymer manufacturing plants represents a unique microbial environment, so if one compares it to municipal landfill sites, the soil here has been constantly exposed to plastic waste. This means that fungus which can consume LDPE as a carbon and energy source may dominate the soil. However, not too many studies have been conducted to examine this environment (Sangaleet *al.*, 2019). The present study was therefore undertaken with the following objectives: to study the native fungal flora in the soil of the manufacturing waste yard and compare it with other locations; to measure its LDPE-destroying ability using qualitative and quantitative methods; to characterize the chosen fungi. The results of the research should assist in the development of a more efficient way of removing plastic.

REVIEW OF LITERATURE

Fungal Degradation of LDPE

Among the organisms that degrade low-density polyethylene (LDPE), fungi appear to be particularly common. According to Sangaleet *al.* (2019), 109 fungi were screened from regions in mangrove forests located near landfill sites on India's western coast, resulting in the isolation of cultures capable of degrading LDPE and reducing its film mass by more than 58% after two months and its tensile strength by more than 94%. The efficacy of the various culture types varied greatly depending on the pH of the incubation. Several fungi have been isolated and reported as decomposers of LDPE, including strains of *Trichoderma harzianum* that were isolated from domestic wastes by Sowmyaet *al.* (2014) using electron microscopy technology that

allowed for the identification of the organism's ability to degrade LDPE.

As fungi in the genus *Aspergillus* have continuously been noted as excellent degraders of LDPE, Gajendiranet *al.* (2016) isolated *Aspergillus clavatus* strain JASK1 from landfill soil and confirmed its identification using molecular biological techniques. The researchers claimed to have seen a loss of LDPE mass by 35% after 90 days in the culture with accompanying degradation symptoms seen in electron microscopy analysis. Most recently, Rojas-Villacortaet *al.* (2025) reported the identification of three different indigenous *Aspergillus* strains - *A. niger* H1C, *A. ochraceopetaliformis* H3C, and *A. tamarii* H6C isolated from polluted soils in Trujillo, Peru with evidence on weight loss of LDPE equal to $4.25 \pm 1.67\%$ for the first strain and $3.79 \pm 1.52\%$ for the second strain. Over a period of 50 days, at least some recent studies corroborate the previous role of *Aspergillus* and similar genera in degrading LDPE, while also encompassing a larger group of fungal species involved in this process. Ahmed *et al.* (2025) determined that under laboratory conditions, *Aspergillus niger* caused $45.62 \pm 0.21\%$ loss of LDPE weight in liquid medium after a month compared to $36.0 \pm 0.21\%$ for *Trichoderma harzianum* under similar circumstances. Again, lower losses were measured for both fungi in the soil test. Khan *et al.* (2023) isolated *Penicillium citrinum* from the soil of a plastic waste disposal site in Bhopal and demonstrated its ability to degrade LDPE thus further extending the use of microbial degraders encountered in soil to those beyond *Aspergillus*. Fungi isolated from the plastisphere were studied by Sathiyabamaet *al.* (2024) who identified *Cladosporium sphaerospermum* as a successful organism able to degrade LDPE with an impressive efficacy of a 15.23% loss in weight in just one week. Finally, Yang *et al.* (2024) illustrated that a mixed consortium of

Alternaria sp. and *Trametes* sp. obtained from landfills was able to degrade LDPE in an effective way.

Mechanisms, Screening Tests, and Confirmation Methods

Literature reviews conducted in the area reveal that fungi decompose polyethylene according to similar mechanisms: the first step is colonization and biofilm formation on polyethylene (biodegradation) followed by the cleavage of the polymer chain (biofragmentation) which then results in the absorption of the resulting oligomers and monomers (Restrepo-Flórez *et al.*, 2014; Sen & Raut, 2015; Ru *et al.*, 2020). Such enzymes as laccase and manganese peroxidase as well as hydrolases like lipases and esterases are deemed responsible for the cleavage of the polymer chain (Sowmya *et al.*, 2014; Sangaleet *et al.*, 2019). Screening tests are mainly performed in a clear zone (or halo) assay using mineral salts agar, LDPE powder follows (Gajendiran *et al.*, 2016; Rojas-Villacorta *et al.*, 2025).

MATERIALS AND METHODS

Study Area and Soil Sample Collection

Soil samples were gathered from seven different sites located in Modinagar, Ghaziabad, and Meerut in the Indian state of Uttar Pradesh. Locations for sampling included waste disposal of companies engaged in polyethene and polymer manufacturing and nearby roads on which polythene waste was also accumulated. At each site, surface soil (approximately 0–15 cm depth) was collected using a sterile scoop into sterile polyethylene-free sample bags, labeled, transported to the laboratory under ambient conditions, and processed within 24–48 hours or stored at 4 °C until use. Table 1 summarizes the sampling locations and the nature of the material collected at each site.

Table 1. Soil sample collection sites

Location name	Sample collected
Krishna Nagar, Modinagar	Soil sample
Sonda Road, Modinagar	Soil sample and waste polyethylene
Raj Bala, Modinagar	Soil sample and waste polyethylene
Compound southside industrial area (polyethylene industry), Ghaziabad	Soil sample and polyethylene waste
Subharti University ground, Meerut	Soil sample
Mariam Road, Ghaziabad	Soil sample and polyethylene waste
Shihani Road, Ghaziabad	Soil sample and polyethylene waste

Isolation of Indigenous Fungi

One gram for each sample of the soil was dissolved in sterile saline using sodium chloride concentration as 0.9 which is further serially diluted in concentrations of ten raised to minus one to ten raised to minus ten. Appropriate dilutions of the aliquots were spread plated on potato dextrose agar and Czapek-Dox agar along with supplemented antimicrobial agents to minimize the growth of non-fungal contaminating species in the agar. The incubation of the fungi occurred at the temperature 25°C-30°C for period of three to seven days; colonies that had a distinct morphology were selected from the PDA plates and were repeatedly subjected to culturing on fresh PDA. The process was broadly similar to those that deal with LDPE degrading species from soil (Sangaleet *et al.*, 2019; Gajendiran *et al.*, 2016; Rojas-Villacorta *et al.*, 2025). Enrichment cultures were also made by using mineral salt media with LDPE powder or film with sub-culturing done at intervals of 10 to 15 days for enriching fungal species that degrade LDPE.

Initial Screening of Fungus

Initial screening of fungal species was performed by using the clear-zone method. Pure fungal species were inoculated onto the MSM medium along with different types of LDPE to check their growth. The resulting clear zones were stained using Coomassie

Brilliant Blue R250 to see which of the fungi were able to grow and produce a halo.

Secondary Screening of LDPE

Weight of LDPE was obtained prior to three procedures so that it could be sterilized. Three factors help the fungi to grow in the MSM broths and check if the fungi that produce high halos are capable of degrading LDPE. Measurements taken at the end of incubation showed that films could be washed and weighed to demonstrate the results.

Study of morphology of fungus

Fungal isolates with the maximum degradation of LDPE were examined for colony morphology characteristics on PDA or Czapek-Dox agar. The isolates were also subjected to a slide-culture method to study the microscopic features of them.

RESULTS

Isolation and Primary Screening of Fungal Isolates

In total, there were 17 fungus isolates recovered with notable colony formations from the seven different sampling sites and out of which the sites concerning polyethylene-sector waste-yard plus roadside showed even more or fewer number of morphologically distinctive fungi as compared to the reference sites with no polyethylene exposure. Out of them, 4 isolates had a visible zone of clearance in LDPE-supplemented mineral salt agar after Coomassie Brilliant Blue staining and they went through subsequent quantification (Table 2).

Table 2. Primary screening — fungal isolates showing a zone of clearance

Isolate code	Source location	Zone of clearance (mm)
Isolate F1	Shihani Road, Ghaziabad	47.5
Isolate F2	Sonda Road, Modinagar	32
Isolate F3	Raj Bala, Modinagar	18
Isolate F4	Mariam Road, Ghaziabad	13

Morphological Characterization of the Dominant Fungal Isolate

The isolation of fungus present in the waste yard of the polyethylene industry showed the formation of rapidly growing colonies on Czapek–Dox/PDA agar. Microscopic observations (LPCB wet mount, bright-field microscopy) revealed many actively growing erect, smooth walled, unbranched septate conidiophores originating from a surface of substrate, all of which ended in swollen, rounded vesicles (Figures 1-3). Biseriatephialides covering upper two-thirds of the vesicle or the whole surface of the vesicle produced such long chains of conidia in a radial manner forming the structure of the conidial head. Packed conidial heads appeared dark brown in color, while the younger heads looked less dark colored (Figure 3 upper head), which is a normal pattern in the maturation process of conidia.

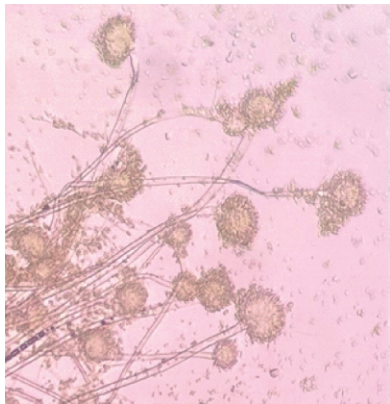


Figure 1. Low-power photomicrograph of the mycelial mat of the dominant fungal isolate recovered from LDPE-enrichment culture, showing numerous erect conidiophores terminating in radiate, biseriate conidial heads at varying stages of maturity (bright-field microscopy, LPCB wet mount).

The features seen in the specimen (like smooth, unbranched, septate conidiophore emerging from the foot cell; globular vesicle; biseriate fashion of phialide and dark brown color of conidia) suggested possible classification of the specimen into the genus

Aspergillus. In literatures, the genus *Aspergillus niger* has been reported to show strong adaptability to LDPE in polymer polluted areas (Gajendiranet *al.*, 2016; Rojas-Villacortaet *al.*, 2025).

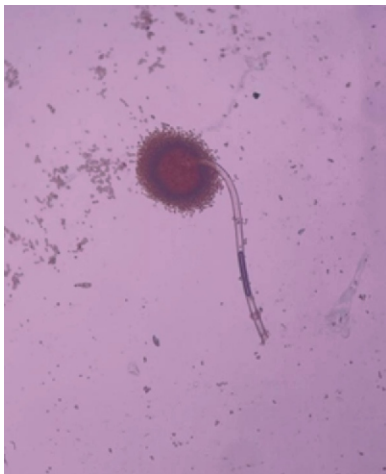


Figure 2. Photomicrograph of a single mature conidial head of the dominant isolate, showing a globose vesicle bearing radiating biserial phialides and chains of dark-pigmented conidia, borne on a long, smooth-walled, septate conidiophore.

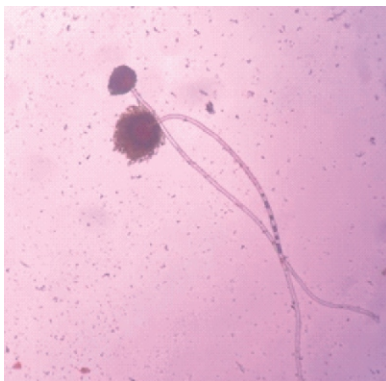


Figure 3. Photomicrograph showing two conidial heads at different stages of maturation - a smaller, less densely pigmented immature head and a larger, darker mature head - on unbranched, septate conidiophores arising from the substrate mycelium.

LDPE Weight-Loss (Biodegradation) Assay

Isolates that were chosen according to the outcomes of the primary screening are to be assessed for their capability to achieve weight

loss in LDPE films throughout the incubation period. The film without inoculation is presumed to remain unchanged regarding its weight during the experiment (Table 4 where the isolates are going to be defined with the results obtained in the current investigation). For better understanding, Table 3 shows sample data on weight loss in selected fungal strains (primarily *Aspergillus* species) obtained in similar research works – it serves as a reference regarding weight loss results achieved in our investigation.

Table 3. Representative LDPE weight-loss values reported for fungal isolates in comparable published studies (literature benchmark)

Fungal isolate	Isolation source	Incubation period	Weight loss (%)	Reference
<i>Aspergillus niger</i> H1C	Plastic-contaminated soil, Peru	50 days	4.25 ± 1.67	Rojas-Villacortaet <i>al.</i> (2025)
<i>Aspergillus tamaraii</i> H6C	Plastic-contaminated soil, Peru	50 days	3.79 ± 1.52	Rojas-Villacortaet <i>al.</i> (2025)
<i>Aspergillus clavatus</i> JASK1	Landfill soil, India	90 days	~35	Gajendiranet <i>al.</i> (2016)
Mixed fungal isolates	Mangrove rhizosphere/dumping site, India	60 days	up to ~58	Sangaleet <i>al.</i> (2019)

Table 4. LDPE film weight loss (%) for isolates of the present study after 30/60 days of incubation

Isolate code	Weight loss at 30 days (%)	Weight loss at 60 days (%)	Clear-zone diameter (mm)
Isolate F1	18.79 ± 1.5	42.54 ± 1.5	47.5
Isolate F2	13.45 ± 1.5	29.29 ± 1.5	32
Isolate F3	11.53 ± 1.5	23.61 ± 1.5	18
Isolate F4	10.94 ± 1.5	21.82 ± 1.5	13

Colony and Microscopic Morphological Summary

Table 5 summarizes the colony and microscopic morphological characteristics recorded for the fungal isolates advanced through screening, including the tentative genus-level identification supported by the photomicrographic evidence presented in Section 4.2.

Table 5. Colony and microscopic morphological characteristics of the most active LDPE-degrading fungal isolates

Isolate code	Colony morphology	Microscopic morphology	Tentative identification
Isolate F1, Figs. 1-3	Fast-growing, granular, dark brown-to-black colony on Czapek-Dox/PDA	Unbranched septate conidiophore; globose vesicle; biseriate phialides; dark brown-to-black radiate conidial chains	Aspergillus sp. (A. niger aggregate) - tentative, morphology-based

DISCUSSION

The polymer production site waste area indicates chronic exposure to polyethylene because it is totally different from the publicly available landfill and municipal solid waste disposal sites from which most previously noted low-density polyethylene-degrading fungal species were taken (Gajendiranet al., 2016; Rojas-Villacortaet al., 2025). Because the impact of those areas brings constant selective pressure in polymer utilization, it can be assumed that they contain fungi that can use LDPE as the carbon source, which agrees with the data obtained about other polymer-contaminated environments like mangrove rhizosphere soil at the dumping sites (Sangaleet al., 2019).

The data obtained photomicrographically in this work (Figures 1-3) is consistent with the well-known fact from literature that black-spored Aspergillus fungi, including A. niger fungus, are good colonizers of LDPE found in the soils of the polymer-contaminated environment (Gajendiranet al., 2016; Rojas-Villacortaet al., 2025). The structure of the conidial head of *Aspergillusniger* and color of its conidia match the description of the genus shape noted in the literature, thus providing evidence for the latest genus identification; however, molecular confirmation is still required for proper identification of the species. The screening method developed for this research — clear-zone qualitative screening followed by quantitative weight-loss and pH monitoring — conforms with the methodology used in several high-quality

recent studies of fungal LDPE biodegradation (Rojas-Villacortaet al., 2025; Gajendiranet al., 2016) and is aimed at eliminating the risk of confusing leaching of additives and abiotic oxidation with actual biodegradation, as noted by Montazeret al. (2020).

The LDPE biodegradation extent varies greatly between studies, from less than 5% in 50 days for separate species from Aspergillus genus in vitro (Rojas-Villacortaet al., 2025) to over 35% for *Aspergillus clavatus* in 90 days (Gajendiranet al., 2016), or even more than 58% of the total weight lost for mixed fungal cultures from mangrove rhizosphere soil in 60 days (Sangaleet al., 2019) and 45.62 ± 0.21% for *Aspergillus niger* in 30 days in liquid medium (Ahmed et al., 2025) and even 15.23% in only 7 days for the quick isolate *Cladosporiumsphaerospermum* (Sathiyabamaet al., 2024). This points to the need to interpret the measurements from this research comparatively and to look whether or not the evidence for weight loss is consistent in replications rather than unique and whether it was supported by clear morphological evidence corresponding to the identification applied.

It is important to mention some drawbacks of this kind of study: lab testing, even if it lasts for 60 days, is nothing when compared with decade wide, if not century winding lifespan of LDPE in the environment, as demonstrated by the half-life exceeding 890 days for fast LDPE decomposing fungus Aspergillus species tested in laboratories (Rojas-Villacortaet al., 2025).

CONCLUSION

The goal of this investigation was to bioprospect the soil near a polymer manufacturer’s waste yard, as well as comparator sites in Modinagar, Ghaziabad, and Meerut, for any native fungi that can break down low-density polyethylene. By using a multi-step protocol for isolation and screening — enrichment culture, clear-zone screening,

quantitative weight-loss assay, and morphological identification — this study creates the basis for the selection of promising fungal isolates that could be processed further. Observation of the characterized dominant isolate obtained from the waste-polymer soil showed that it had radially biseriate conidial heads with dark brown to black conidial pigmentation consistent with the fungi belonging to the *Aspergillus* genus (specifically, the *A. niger* aggregate), which confirms previous scientific works showing that *Aspergillus* is widely spread LDPE colonizer found in polymer-contaminated soils too. The soil in industrial waste yards that has been exposed to polyethylene for a long time appears to be a promising area with not enough studies conducted, so the isolates obtained might be used in the future for bioremediation plans and recycling techniques and methods, while the next step would be molecular identification and spectroscopic or microscopic confirmation of degradation processes.

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Received : January 2026; Revised : February 2026; Accepted : March 2026



STANDARDIZATION OF DIFFERENT CONCENTRATION OF IBA & NAA ON COMMERCIAL PROPAGATION METHOD OF LITCHI (LITCHI CHINENSIS SONN.)

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Abstract

The study was conducted at the Horticultural Research Centre, College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.). with the objectives to standardize the most effective concentration and combination of IBA and NAA for commercial propagation of litchi by air layering. The experiment was laid out in a Randomized Block Design with nine treatments, including IBA, NAA, and their combinations. Among the different treatments evaluated, the combined application of IBA 2000 ppm + NAA 2000 ppm (T9) emerged as the most effective across multiple parameters. Physiologically, this treatment resulted in the earliest root initiation (22.0 days), the highest number of primary roots (21.3) and secondary roots (29.3), the longest root length (7.90 cm), and the maximum root thickness (1.87 mm), indicating superior rooting efficiency. It also recorded the highest rooting percentage (80.0%), while reducing the days to initiation of new leaves (15.0 days), thereby accelerating establishment. In terms of vegetative performance, T9 achieved the maximum total number of leaves (18.0) and the highest survival percentage after transplanting (85.0%), while recording the lowest mortality percentage (10.0% at 60 DAT). The mean performance of all IBA + NAA combined treatments was superior to IBA or NAA alone, confirming their synergistic effect in promoting rooting and survival. Overall, the study clearly demonstrated that the combined application of IBA 2000 ppm + NAA 2000 ppm was the most effective treatment for improving rooting success, survival percentage, and overall growth of litchi air layers under the conditions of western Uttar Pradesh. This treatment offers a practical and reliable propagation method for commercial litchi cultivation, ensuring higher survival, reduced mortality, and vigorous planting material for orchard establishment.

Keywords: ppm, DAT, IBA, NAA etc.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is a subtropical evergreen fruit valued for its flavour and rich nutritional content, primarily grown in China and India, which together contribute 91% of world production. Despite its popularity, litchi cultivation faces constraints like specific climatic needs, poor fruit set, and limited regional cultivars. Litchi likely originated in Southern China, spreading to India by the 18th century. China and India are the largest producers globally; Bihar leads Indian production, accounting for 74% of national output. The fruit is appreciated for its sweet-sour taste, high vitamin C (up to 71 mg/100g), and a variety of culinary uses such as juices, jams, and ice cream. Commercial propagation relies on air layering, a technique challenged by low nursery survival and unreliable rooting. The use of plant growth regulators—especially auxins like IBA (indole-3-butyric acid) and NAA (Naphthaleneacetic acid)—has shown effectiveness in promoting rapid, uniform root development, although optimal concentrations vary by species and conditions. Research aims to optimize IBA and NAA use in propagation to improve survival, reduce mortality, and enhance cost-effectiveness for litchi growers. Plant growth regulators, particularly auxins like Indole-3-butyric acid (IBA) and Naphthaleneacetic acid (NAA), play a pivotal role in enhancing root initiation and development in litchi air layers. Previous studies have indicated that appropriate concentrations of IBA and NAA can significantly increase root length, number of roots, and overall survival of air layers. However, the response varies with species, concentration, and environmental conditions, necessitating precise standardization. The present research aims to standardize the concentrations of IBA and NAA for efficient commercial propagation of litchi. The study

focuses on determining optimal hormone levels to achieve vigorous root development, reduce mortality in nursery conditions, and produce true-to-type, high-quality planting material. The findings are expected to enhance the success rate of vegetative propagation, ensure uniform plant production, and contribute to the sustainable expansion of litchi cultivation. Ultimately, optimizing the use of plant growth regulators can make litchi propagation more economical and reliable, benefiting both growers and the horticultural industry.

MATERIAL AND METHOD

The present experiment was conducted during 2024–25 at the Horticultural Research Centre, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh, India (latitude 28°57'–29°02' N, longitude 77°40'–77°45' E, elevation 237.75 m), under a subtropical climate with annual rainfall of 800 mm and occasional winter frost. The research focused on optimizing propagation of litchi (*Litchi chinensis* Sonn.) using different concentrations of IBA and NAA plant growth regulators under western Uttar Pradesh conditions.

The study used a Randomized Block Design (RBD) with nine treatments (control, IBA 1000 ppm, 2000 ppm, NAA 1000 ppm, 2000 ppm, and combinations IBA 1000 ppm + NAA 1000 ppm, IBA 1000 ppm + NAA 2000 ppm, and IBA 2000 ppm + NAA 1000 ppm, IBA 2000 ppm + NAA 2000 ppm) and three replications, with five layers per replication (total 135 plants). Air layering was performed on healthy one-year-old terminal branches at the start of July using the respective PGRs in lanolin paste, and branches were wrapped with moistened sphagnum moss and polyethylene. Roots were assessed after visibility through polyethylene; layers were detached when well-rooted.

Table 1: Illustration about treatment and treatment symbol

S. No.	Treatments	Treatments Symbol
1.	Control (no concentration)	T ₁
2.	IBA 1000 ppm	T ₂
3.	IBA 2000 ppm	T ₃
4.	NAA 1000 ppm	T ₄
5.	NAA 2000 ppm	T ₅
6.	IBA 1000 ppm + NAA 1000 ppm	T ₆
7.	IBA 1000 ppm + NAA 2000 ppm	T ₇
8.	IBA 2000 ppm + NAA 1000 ppm	T ₈
9.	IBA 2000 ppm + NAA 2000 ppm	T ₉

Observations recorded

(a) Days taken for root initiation

The number of days taken for appearance of first roots in air layers, which were visible through the transparent polythene sheet was recorded by periodic observation.

(b) Number of Primary roots

The mean number of primary roots per layers was calculated from the total number of primary roots of all four layers.

(c) Number of Secondary roots

The mean number of secondary roots per layers was calculated from the total number of secondary roots of all four layers.

(d) Length of roots (cm)

The mean maximum length of roots was calculated from the total number of air layers and expressed in cm.

(e) Thickness of roots (mm)

Root thickness was taken on the basis of the thickness of root acquired by all the roots from the selected rooted layers.

(f) Rooting percentage (%)

Rooting Percentage
$$= \frac{\text{Total number of rooted air layers}}{\text{Number of treated air layers}} \times 100$$

(g) Days to initiation of new leaves

The number of days from the date of

transplanting to the appearance of visible leaf was recorded under each treatment in days.

(h) Total number of leaves

Number of leaves of each of the five randomly selected per plants was counted and recorded.

(i) Survival percentage (%) of plant after transplanting

Survival percentage
$$\frac{\text{Total number of rooted air layered survived}}{\text{Total number of rooted air layered planted}} \times 100$$

(j) Mortality (%)

After transplanting of air-layered plants in open field, observation on mortality was recorded at 15 days interval up to 60 days. It was calculated by the following formula and expressed in percent.

Mortality (%)
$$\frac{\text{Total number of plants died}}{\text{Total number of layered plants planted}} \times 100$$

RESULT AND DISCUSSION

The present investigation evaluated the influence of different concentrations and combinations of the plant growth regulators IBA and NAA on litchi propagation through air layering. The study was conducted at the Horticultural Research Centre, Modipuram, under the aegis of Sardar Vallabhbhai Patel University of Agriculture and Technology during 2024-25. Results revealed that combined applications of IBA and NAA significantly enhanced all measured propagation metrics compared to single applications or controls.

The combination treatment (T9, IBA 2000 ppm + NAA 2000 ppm) produced the most favourable outcomes, including the shortest

time to root initiation, greatest number of primary and secondary roots, longest and thickest roots, highest rooting and survival percentages, most leaves, and the lowest mortality rates at different intervals after transplanting. The synergistic effect of the combined treatments consistently outperformed individual applications of both auxins in every critical parameter, confirming the effectiveness of using IBA and NAA together for litchi propagation.

Table 2: Effects of IBA and NAA Treatments on Litchi Propagation

Parameter / Treatment	T ₁ (Control)	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
Days taken for root initiation	29.00	25.60	25.00	26.00	25.30	24.00	23.00	22.30	22.00
Number of primary roots	12.0	17.0	18.0	15.0	16.0	17.0	18.0	20.0	21.3
Number of secondary roots	20.0	23.0	24.6	22.6	24.0	25.0	27.0	28.0	29.3
Length of roots (cm)	5.46	7.06	7.50	6.46	6.67	6.90	7.34	7.67	7.90
Thickness of Root (mm)	0.70	1.17	1.37	1.10	1.27	1.47	1.57	1.77	1.87
Rooting percentage (%)	55.0	72.0	75.0	70.0	72.0	74.0	76.0	78.0	80.0
Days to initiation of new leaves	19.0	17.3	17.0	18.0	17.7	17.0	16.3	15.3	15.0
Total no. of leaves	8.6	11	14	12	13	15	16	17	18
Survival (%) of plant after transplanting	60.0	73.3	80.0	66.6	73.3	80.0	83.4	84.0	85.0
Mortality % at 15 DAT	50.0	38.0	30.67	42.0	34.33	28.0	24.33	24.67	17.67
Mortality % at 30 DAT	44.0	30.67	23.33	38.0	30.0	24.0	20.0	20.0	14.0
Mortality % at 45 DAT	40.0	26.7	20.0	33.4	26.7	20.0	16.6	16.0	15.0
Mortality % at 60 DAT	42.0	28.0	19.0	36.0	27.67	18.33	14.0	15.0	10.0

CONCLUSION

The results of the present study clearly indicate that the application of plant growth regulators, particularly IBA and NAA, has a significant effect on rooting, vegetative growth, survival, and establishment of litchi air layers. Among all treatments, the combined application of IBA 2000 ppm + NAA 2000 ppm (T9) consistently outperformed all other treatments across all recorded parameters. Therefore, it can be concluded that the combined application of IBA 2000 ppm + NAA 2000 ppm is the most suitable treatment for commercial propagation of litchi by air layering under the agro-climatic conditions of western Uttar Pradesh. This practice not only improves rooting and establishment success but also reduces mortality, thereby ensuring higher survival of

planting material, which will greatly benefit litchi growers by providing true-to-type, healthy, and vigorous plants for orchard establishment.

ACKNOWLEDGEMENT

The author expresses sincere gratitude to the Department of Fruit Science, College of Horticulture, SVPUAT, Meerut, for providing the necessary facilities and support to carry out this research work. Heartfelt thanks are extended to my research advisor, Dr. [Satya Prakash], for valuable guidance, constructive suggestions, and constant encouragement throughout the study.

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Received : January 2026; Revised : February 2026; Accepted : March 2026



AGROBIOTECHNOLOGICAL POTENTIAL OF *PLEUROTUS OSTREATUS* FROM AN ECOLOGICAL–SOCIOECONOMIC PERSPECTIVE IN INDIA: A REVIEW

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Abstract

India produces nearly 1 billion tonnes of agriculture residues and agro-industrial waste each year, much of which is disposed of via open field burning, resulting in seasonal air pollution crises, nutrient loss from soil, and greenhouse gases into the atmosphere. In light of this problem, the white-rot fungi *Pleurotus ostreatus* (oyster mushrooms) can provide a unique coupling of ecological remediation, food security, and rural job creation. The present paper reviews the available published literature (2020-2026) to explore the agro-biotechnology potential of *P. ostreatus* via an integrated ecological and socio-economic perspective (specific to India). Published literature is reviewed related to the biology of *P. ostreatus* as well as descriptions of the low input production systems that grow the mushroom; substrate types that can be used to grow the mushroom; and descriptions of the ligninolytic enzymes involved in the decomposition of lignocellulosic substrates and degradation of environmental toxic compounds (i.e. dyes, polycyclic aromatic hydrocarbons), such as other heavy metals. Nutritional literature, describing protein, dietary fibre, β -glucans, and phenolic compounds, is reviewed; and examples of hypolipidaemic, antioxidant, and immunomodulatory effects of edible fruiting bodies (mushrooms) are provided. Finally, examples of both mushroom substrate valorisation and the development of mycelium biomaterials to replace plastic and synthetic materials in a circular bioeconomy are presented. The socioeconomic aspect of Indian smallholders shall be measured by evaluating *P. ostreatus* with regards to employment, empowerment of females, self-help group business enterprises, and tribal/homestead livelihoods. The review concludes that *P. ostreatus* presents a strategic opportunity for sustainable agricultural production in India. However, three main issues impacting the full potential of *P. ostreatus* must be addressed: quality of spawn, post-harvest handling systems; and standardisation and/or support from policy.

Keywords: *Pleurotus ostreatus*, oyster mushroom, lignocellulosic waste valorization, mycoremediation, circular bioeconomy, rural livelihoods, women empowerment, India.

INTRODUCTION

India has a significant agrarian workforce, but the amount of agricultural waste produced from agriculture every year is overwhelming to the extent that it can be considered more than a nuisance. However, there is great potential for mushroom cultivation to recycle an immense portion of approximately 947 million metric tons of agro-waste created in India each year- thereby establishing mushroom cultivation as a possible agricultural successor to the Green Revolution for both resource efficiency and impact on rural areas (Thakur et al., 2025). Of all edible fungi cultivated, *Pleurotus ostreatus* or “oyster mushroom” is one of the most suited for cultivation in that it can be grown using a range of agricultural waste materials without supplementing; mushrooms require little capital or infrastructure; *Pleurotus ostreatus* can complete its growth cycle within 3–4 weeks of seed placement and produce a nutritious edible product readily available for sale.

As the third most planted type of fungus across the world, the species *P. ostreatus* grows in numerous places, making it the second most important edible fungus grown commercially throughout all of India after the white button mushroom. In India, combined sales of oyster, paddy straw, and milky mushrooms account for an increasingly larger percentage of overall sales in this country (Bijla & Sharma 2023; Thakur et al. 2025). There are three interrelated bases of attraction for *P. ostreatus*: (1) it destroys wood fibers and detoxifies chemicals with special enzymes to make contaminated waste food-safe and bio-remediate polluted ecosystems, (2) it provides protein, fiber, antioxidants, and many other health-promoting nutrients in addition to other healthy plant compounds and chemical constituents that are considered to be safe, and (3) it is a low-cost way for individuals

(especially women, landless immigrants, and members of tribal groups) to gain a source of income (Patel et al. 2023; Thakur et al. 2025).

The purpose of this review is to summarize research published from 2020 through 2026 on the agrobiotechnological potential of *P. ostreatus* from an ecological-socioeconomic perspective based on conditions in India. The review will cover various topics: morphology and growth of the fungus; the enzymes involved in lignocellulolysis and degradation of pollutants; nutritional and medicinal benefits to humans; mycoremediation; circular bioeconomy; and socioeconomic contributions. Finally, the review will discuss the major obstacles and research needs that must be addressed in order to increase the use of *P. ostreatus*.

Taxonomy, Biology and Cultivation Systems in India

Biology and ecological niche

Dhingri is the common name in India for *Pleurotus ostreatus*, which is a type of basidiomycete (fungus) that belongs to the Pleurotaceae family. It is also known to exist in nature as a saprophytic (living off dead or decaying matter) and facultative (can live on either decaying or fresh food) white rot (fungus that decomposes wood and produces white, mycelium) fungi, which can produce mushrooms on dead/decaying hardwood trees using enzymes to break down the cellulose and hemicellulose within the wood to use as food sources for carbon and energy. The fruiting body of *P. ostreatus* has a shell or pair shape, a very short and commonly irregular or lateral, and gills that produces the basidiospores (spore producing structure of fungi). The species will grow within a relatively large temperature range of between 20C to 30C with a relative humidity of 55-185% at which point it has been grown throughout most of the Indian plains and has the ability to grow (with

humidity controls), on the hilly areas of India (Thakur et al. 2025). So with rapid mycelial colonisation and this ability to thrive over a wide range of climatic conditions (temperature and humidity), *P. Anathema* is considered one of the most robust (hardy), beginner friendly edible mushrooms for smallholder farmers to cultivate.

Substrates and low-input cultivation

The oyster mushroom is an important plant in the agriculture biotechnology business because it is able to grow on many different types of unused agricultural residue products. This ability allows agriculture farmers to tie the use of these products to waste management. Studies in India compared the growth of mushrooms on different substrates such as wheat straw, paddy straw, sawdust, sugarcane residual, coconut husk, millet straw and banana residue found that the mushrooms produced on different substrates resulted in a different crop substrate potential; sawdust and sugarcane residual generated fast spawn run, and the growing of the oyster mushrooms in Tamil Nadu produced high weight crops (Sathiyaseelan et al., 2024). Dry sawdust was also found to be an effective substrate for growing mushrooms in northern India (Negi et al., 2022), while researchers in southern India developed materials and methods for growing *Pleurotus* species in greenhouse or shed-type units and low-cost packaging (Deora et al., 2021).

Substrate pasteurization, cropping, and fruiting phases occur within a cultivated environment that provides controlled humidity and ventilation. Inputs for cultivation are generally inexpensive (crop residue) and facilities can often be improvised (small household to multi-storey commercial units). There is a large number of small producers planting in Purba Bardhaman, West Bengal during the cooler months, for example; several hundred women produce

oyster mushrooms through polybag culture at this time of year (Thakur et al, 2025). The ecological value of the substrate versatility that enables ease of production is therefore the same substrate that will go to waste or be partially de-lignified through fungal metabolism at the completion of the cropping process (Melanouri et al, 2022).

Lignocellulosic Waste Valorisation and Enzymatic Machinery

The ability of *P. ostreatus* to colonize agricultural residues is a direct result of its extracellular enzyme system. This fungus produces a complementary set of oxidative ligninolytic enzymes—principally, laccases, manganese peroxidase and lignin peroxidase—as well as hydrolytic cellulases and hemicellulases (e.g., endoglucanase, xylanase). The synergistic relationship between oxidative and hydrolytic enzymes allows the fungus to depolymerise and partially mineralise lignin in order to access cellulose or hemicellulose for consumption (Lozada Martinez et al., 2026). As laccases play a key role in lignin oxidation and the large number of different types of substrates, their production can be optimised by using different substrate types and applying proper conditions of solid-state fermentation (Melounouri et al., 2022).

When oyster mushrooms are cultivated on lignocellulosic materials, they are selective in their removal of hemicellulose and lignin, leaving a residue that is enriched in cellulose. This transformation not only produces biomass that can be consumed, but also prepares the substrate for subsequent usage. In addition to generating food for humans, selective delignification has other important uses: it converts hard-to-digest residuals into more easily digested livestock feed; it creates feedstock that can be further converted into fuel; and it produces organic matter that is partially stabilized (Lozada-Martinez et al.,

2026; Melanouri et al., 2022). For India, where there is an abundance of wheat straw and it is often disposed of by burning, the enzymatic valorisation of these materials by *P. ostreatus* represents a biologically-based alternative to producing both food and reducing open-field burning. Furthermore, *P. ostreatus* has been studied as a beneficial bioinoculant; its mycelia can enhance growth of plants and suppress wilting due to *Fusarium oxysporum* (Sarkar & Datta, 2024), showing that the biotechnological footprint of *P. ostreatus* is found both in waste-to-food conversions and plant health applications.

Nutritional and Nutraceutical Value

Oyster mushroom food value contributes to both marketable needs and provides nutritional security. Their fruiting bodies have low levels of fat and calories, contain very high-quality proteins that are highly complete in terms of essential amino acids while providing dietary fiber, vitamins A & B, minerals, etc.; also, because of the generally low biological value of mushroom protein compared to traditional animal foods, it should be noted that the biological value is not only higher than all cereal and pulse sources combined, but also higher than both animal protein and dairy protein sources (Thakur et al., 2025). The main functional group of components would be the polysaccharides found in the cell walls of mushrooms, specifically the β -glucan fraction, as well as phenolic compounds, ergosterol and other phytochemicals present that provide antioxidant properties (Effiong et al., 2024).

Phenolic content, radical scavenging ability and reducing potential were all confirmed in a number of comparative analyses of extracted fruiting body and mycelium of *Pleurotus ostreatus*, demonstrating that the tasty edible mushroom exhibits antioxidant properties

and should therefore be classified as a functional food (Effiong et al., 2024). β -glucans are of particular interest due to their potential to enhance immune function and serve as prebiotics, and extraction studies indicate that new assisted extraction techniques can yield improved quantities and/ or bioactivity of these compounds compared to traditional aqueous or ethanolic extraction methods; this information is also applicable to product development from both the fruiting body and the mycelium (Frioui et al., 2024). Oyster mushrooms can also be produced more economically on waste substrates than any other source of plant protein while providing highly nutritious food for the large number of vegetarians in Indian culture who depend on plant proteins in their diet.

Medicinal and Bioactive Potential

P. ostreatus is an excellent source of nutraceuticals and pharmacologically active compounds, in addition to its basic nutritional properties. β -glucans, phenolic acids, ergothioneine, lectins, and sterols found in *P. ostreatus* demonstrate antioxidant, immune-modulating, anti-inflammatory, cholesterol-and lipid-lowering, and hypolipidaemic and anti-hyperglycaemic effects (Frioui et al., 2024). The most frequently indicated pharmacologic property of *P. ostreatus* is its cholesterol- and lipid-lowering effects. The combination of *P. ostreatus* with *Agaricus bisporus* in a controlled animal model showed a reduction in hyperlipidaemia and oxidative stress; research suggests that these improvements are due to the phytosterols, phenolics and different bioactive components present in the mushrooms (Iqbal et al., 2024). Although clinical data are still limited, researchers have seen a growing amount of evidence in favour of *P. ostreatus*. Healthy volunteers consuming an aqueous extraction from *P. ostreatus* displayed

statistically significant changes in blood markers and cytokine levels, suggesting a change in inflammatory and metabolic markers (Dündar et al., 2024). Together with the antioxidant and α -glucan data, these findings support the hypothesis that oyster mushrooms have potential use as dietary agents in the prevention and adjunctive treatment of oxidative stress and metabolic-related conditions. However, it is essential to note the current level of clinical evidence supporting this hypothesis is relatively weak, thus more rigorously designed trials will be required to support any specific therapeutic claims about *P. ostreatus*.

Ecological Dimensions: Mycoremediation and the Circular Bioeconomy

Mycoremediation of pollutants

P. ostreatus is an ideal model organism of mycoremediation, due to its ligninolytic enzymes that can degrade lignin, and its ability to bioremediate a wide range of contaminants; in particular, *P. ostreatus* can oxidise phenolic compounds, synthetic dyes and hydrocarbons using its laccase and manganese peroxydase systems. It also has the capability to grow in and survive in contaminated soil, alongside the indigenous microflora, making it an excellent candidate for use in the field (Lozada-Martinez et al. 2026). A comprehensive evidence-based analysis of the Scopus literature indicates the rapid growth of research into the oxidative transformation of phenolics, dyes and hydrocarbons, as well as the bioconversion of agro-industrial waste products is one of the primary research areas currently being investigated using this species (Lozada-Martinez et al. 2026).

The interaction between *Pleurotus* mushrooms and heavy metals is by biosorption and bioaccumulation.

Experimental evidence indicates that the mushrooms can absorb and withstand metallic cations (copper, cobalt, nickel) with no toxic effects. This gives an inexpensive, environmentally-friendly option to clean up metal contamination in soil and water (Mohamadhasani & Rahimi, 2022). This information has implications for India in two ways: first, it can be used to clean up areas that have been contaminated; second, mushrooms cultivated on contaminated substrates may also accumulate heavy metals; therefore, it is very important to consider the location and quality of substrate for these mushrooms when they are going to be used for food production. Therefore, the potential for remediation could be maximised by using an appropriate remediation application rather than treating the remediation process as a means of producing food.

Spent mushroom substrate and mycelium-based biomaterials

The substrate's useful life is not finished when it is cultivated. Spent mushroom substrate (SMS), leftover after harvest, is a partially delignified, enzyme- and mycelium-rich material that can have value as a soil amendment and bio-fertilizer, as feedstock for additional enzyme or energy recovery, and as a part of a circular bio-economy, rather than being disposed of as waste (Lozada-Martinez et al., 2021). Relying on SMS for reuse creates a loop from residue input to soil return and aligns oyster mushroom production with principles of resource utilization that promote regeneration.

A new application is rapidly growing, utilizing the binding ability of oyster mushroom mycelium to create composite materials made from mycelium. *P. ostreatus* mycelium grows through the lignocellulosic substrate, causing the loose particles to be knitted into a strong yet light-weight material that can be used for packaging and insulation

purposes and also provides a fully biodegradable and low-energy alternative to expanded polystyrene and other types of plastic (Alaneme et al., 2023). Demonstrations are currently existing which include the use of mycoblocks for sustainable construction (Alemu et al., 2022), myco-foams, created using mycelium and reinforced with hemp fibre and formerly discarded peanut shells, which have similar mechanical properties to expanded polystyrene (Picco et al., 2024), and biocomposites, produced using textile residue (waste fabric) and providing another example of how biocomposite products can reduce waste (Saini et al., 2023). In India, where there is a high level of agro-residue and an increasing challenge with respect to packaging-plastics, there is an immediate circular economy opportunity for locally produced mycelium products by leveraging the existing skill base and infrastructure of current mushroom cultivators.

Socioeconomic Dimensions in the Indian Context

There are connections between the social agrobiotechnological opportunities based on the fact that mushroom growing is relatively easy and inexpensive in terms of entry. The cultivation of oyster mushrooms requires a low level of capital investment, a minimum of labor and maximum yield potential that use agricultural by-products, requires minimal area for cultivation and can produce a crop with minimal time to harvest. Therefore, the cultivation of oyster mushrooms is especially well suited to low income people who have little or no access to traditional forms of agriculture, which is otherwise limited in terms of income (Thakur et al., 2025). Research conducted by Shaurma and others (2021) show that growing mushrooms will provide various ways for farmers to make money while providing positive social welfare benefits. As the project scales up from a small scale

production unit to a larger production unit, the economics of mushroom production will increase substantially.

The development of businesses that help women access economic opportunities has been significant because the fact that cultivating mushrooms is a form of agricultural production carried out indoors and compatible with domestic work facilitates women's ability to combine their agricultural productivity with their domestic responsibilities. The skills women in rural areas have developed in mushroom cultivation as an income-generating and self-employment opportunity have been developed through the training they have received from Krishi Vigyan Kendras and Self-Help Groups (SHGs) (Thakur et al., 2025). Also, horticultural interventions have significantly enhanced the economic prospects of women, especially tribal farmers, with mushroom production being one of the productive activities that enhance income and nutrition for women and families in economically disadvantaged communities (Patel et al., 2023). The body of work noted in this paragraph provides a framework for understanding *Pleurotus ostreatus* not only as an agricultural crop, but also as an instrument for rural development that creates jobs for migrant workers, youth who are out of work, and women, while also recycling agricultural waste (Thakur et al., 2025).

Challenges and Constraints

The following are real and significant impediments to the successful actualization of this opportunity in India:

1. There are often unreliable supplies of quality spawn and insufficient quantities of either good planting materials or sufficient quantities of technical assistance and advanced technology; each has placed producers at risk when the flow of inputs and knowledge ceased for an extended period of time due to the COVID-19 pandemic.

2. Because the mushroom crop is so perishable, poor cold-chain, processing and marketing capability results in a great deal of post-harvest loss for smallholder mushroom producers.
3. Competing fungal and bacterial contamination is an ongoing problem throughout the entire mushroom cultivation process; the absence of standardized, locally developed substrate formulations and protocols makes it very difficult to produce consistent yields.

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Additionally, species-related considerations on safety and standardisation apply. High accumulation of heavy metals in *P. ostreatus* makes food-safety concerns over growing conditions of fruiting bodies from contaminated substrate or polluted areas very important when assessing the source and quality assurance of substrates (Mohamadhasani & Rahimi, 2022). In the case of SMS valorisation and mycelium-based products, the other major barriers include a lack of standard processing procedures, quality benchmarks and supporting regulations, as well as logistical issues

associated with aggregating and handling large-scale residues (Lozada-Martinez et al., 2026). Lastly, while promising, there is still not enough clinical evidence to provide valid therapeutic claims for this type of mushroom (Dündar et al., 2024).

Future Perspectives

Clarifying these constraints creates a distinct research/development agenda. The priorities are high-quality spawn with resistance to contamination and an ability to develop/distribute spawn decentralised, as well as developing a substrate within the region that provides the greatest biological efficiency with the residue available in that region (Sathiyaseelan et al 2024); and safely storing (using low cost), providing added value, and connecting smallholder producers with their end-user markets to capture as much of the value chain as possible. Improving strains and optimising the production of ligninolytic enzymes via fermentation engineering will enhance productivity/yield and also the remedial/NOC value of the fungus through remediation and bioconversion (Lozada-Martinez et al 2026; Melanouri et al 2022)

One of the most promising opportunities to use waste as a resource is to grow oyster mushrooms within formal crop-residue management and circular bioeconomy programmes. By doing so, wastage of resources (waste diversion), food production, recycling of SMS and the manufacturing of mycelium-based products can be accomplished through one integrated value chain. In addition, while continuing to provide extension support through Krishi Vigyan Kendras (KVKs) and self-help groups, incorporate mushroom production technology within agricultural and general educational curriculum, and develop and implement policy initiatives that treat SMS and mycelium products as valuable resources, will enhance

the acceptance and adoption of this practice (Thakur et al., 2025). As well as providing ongoing rigorous clinical and toxicological research to facilitate an improved understanding and application of the nutraceutical properties of oyster mushrooms, verification of the documented research will also be critical in terms of developing validated, safe health care applications (Iqbal et al., 2024).

CONCLUSION

In India, *Pleurotus ostreatus* has an extremely important role at the crossroads of ecology, nutrition and rural economies. Its very powerful white-rot enzymes can transform large amounts of lignocellulose waste produced throughout the country and, in many cases, are simply disposed of by burning to be processed into a nutritious food source. The same white-rot enzymes used to convert agricultural waste into a food source are used as the basis for mycoremediation efforts addressing the impacts of dyes, hydrocarbons, and metals, as well as to produce biodegradable mycelium-based products through a circular bioeconomy. As a food source, the fruiting bodies of *Pleurotus ostreatus* provide protein, fiber, β -glucans, and antioxidants, which have been shown to have hypolipidaemic and immunomodulatory effects. Cultivating *Pleurotus ostreatus* requires very few resources and, as a result, provides an income-generating opportunity that can be very beneficial for women, indigenous communities and landless households. Achieving this level of synergy among all three areas of interest (agricultural production, ecological restoration and nutrient utilization) will require coordinated advancements in spawn quality, substrate standardization, available post-harvest infrastructure, food safety assurance and enacted policy supports. Provided that adequate supports are provided, the oyster

mushroom can be utilized as an exemplary model organism for sustainable, inclusive and circular agriculture in India.

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Received : January 2026; Revised : February 2026; Accepted : March 2026



RAD51-MEDIATED DNA REPAIR AND ITS EMERGING ROLE IN PLANT–VIRUS INTERACTIONS: A COMPREHENSIVE REVIEW

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Abstract

Maintenance of genome integrity is indispensable for plant growth, reproduction, and adaptation to environmental stress. Homologous recombination (HR) is a high-fidelity pathway for the repair of DNA double-strand breaks, and the ATP-dependent recombinase RAD51 is its central catalytic component. RAD51 forms a nucleoprotein filament on resected single-stranded DNA, conducts homology search, and promotes strand invasion, while BRCA2, RPA, RAD54, and RAD51 paralogs regulate filament assembly and downstream processing. An emerging body of evidence shows that this repair machinery also participates in interactions between plants and nuclear-replicating DNA viruses, particularly geminiviruses. Because geminiviruses do not encode a complete DNA replication apparatus, they redirect host cell-cycle, replication, recombination, and chromatin factors to convert viral single-stranded DNA into double-stranded intermediates and to amplify viral minichromosomes through rolling-circle and recombination-dependent replication. Experimental studies have implicated RAD54, RAD51D, RAD51, RPA1A, DNA primase, and DNA polymerases α and β in these processes. Conversely, plants can restrict infection by transcriptional immune mechanisms and by altering the genomic distribution of repair proteins. In *Arabidopsis*, BRCA2–RAD51 and SNI1–RAD51D modules connect salicylic-acid-responsive defense-gene transcription with recombination, whereas loss of H3.1K27me1 redistributes RAD51 and RPA1A from viral DNA to unstable host chromatin and defense-associated loci, reducing geminiviral amplification. Recent identification of resistance-associated alleles in host DNA primase and DNA polymerase β further establishes replication proteins as practical targets for crop improvement. This review integrates canonical RAD51 biology with current evidence from plant–geminivirus systems, distinguishes established mechanisms from model-dependent observations, and outlines research priorities for exploiting host repair and replication pathways without compromising genome stability.

Keywords: RAD51; homologous recombination; DNA damage response; geminivirus; RAD51D; RAD54; BRCA2; viral replication; plant immunity

INTRODUCTION

Plants are continuously exposed to DNA-damaging conditions generated by ultraviolet radiation, oxidative metabolism, replication stress, temperature extremes, chemical agents, and pathogen challenge. Among the resulting lesions, DNA double-strand breaks (DSBs) are especially hazardous because an unrepaired or incorrectly repaired break can cause chromosome loss, translocation, mutation, or cell death. Plants repair DSBs mainly through non-homologous end joining and homologous recombination. Non-homologous end joining directly reconnects DNA ends and is active throughout much of the cell cycle, but it can introduce small insertions or deletions. HR uses an intact homologous template and can therefore restore sequence information with high accuracy, particularly in S and G2 phases when a sister chromatid is available (10).

RAD51 is the principal eukaryotic strand-exchange protein in HR. It is structurally and functionally related to bacterial RecA and is conserved across fungi, animals, and plants. Following DSB recognition and end resection, RAD51 assembles on the resulting 3' single-stranded DNA (ssDNA), forms a helical presynaptic filament, searches for a homologous duplex, and catalyzes strand invasion. The reaction is controlled by mediator proteins and paralogs that determine when and where RAD51 is loaded, how long the filament persists, and which recombination outcome is produced. Genetic studies in *Arabidopsis thaliana* and *Physcomitrium patens* have demonstrated both the conservation of RAD51 function and marked lineage-specific differences in the dependence of vegetative cells on HR (7; 9; 1).

The biological significance of RAD51 extends beyond the repair of accidental chromosomal damage. DNA replication, transcription, chromatin regulation, and immune signaling all generate DNA

structures that can recruit repair proteins. Plant pathogens can exploit this integration. The strongest evidence currently concerns geminiviruses, a diverse family of circular ssDNA viruses that replicate in the plant nucleus and cause economically important diseases in crops. Geminiviruses encode a small number of proteins and no complete DNA polymerase; consequently, they depend on host replication and repair activities to establish double-stranded viral minichromosomes and to synthesize progeny DNA (22; 16).

The phrase “plant–virus interactions” in this review is therefore used with an explicit scope: direct mechanistic evidence linking RAD51-family proteins to viral DNA amplification is concentrated in geminivirus systems. It should not be generalized to all plant viruses, most of which possess RNA genomes and replicate through different molecular mechanisms. Within this defined scope, the literature reveals a dynamic competition. Viral proteins recruit host replication and repair factors to viral DNA, whereas plants redirect or regulate the same factors to preserve their own genomes and activate defense. This review synthesizes current evidence for that competition and identifies opportunities and risks associated with manipulating host repair pathways for virus resistance.

Molecular Basis of RAD51-Mediated Homologous Recombination in Plants

DSB recognition, end resection, and presynaptic filament formation

HR begins with detection and processing of a DSB. The broken ends are resected in the 5' to 3' direction to expose 3' ssDNA tails. These tails are initially coated by replication protein A (RPA), which protects ssDNA from nucleolytic degradation and removes

secondary structures. RPA binding is essential but also creates a kinetic barrier to RAD51 assembly. Mediator proteins, most notably BRCA2, promote the replacement of RPA by RAD51 and bias RAD51 binding toward ssDNA rather than undamaged double-stranded DNA. In *Arabidopsis*, BRCA2 supports RAD51- and DMC1-dependent recombination, demonstrating that this core mediator function is conserved in plants (15).

RAD51 monomers polymerize on ssDNA in an ATP-dependent manner to form a right-handed filament. The filament is the active unit for homology sampling and strand exchange. ATP binding stabilizes an active filament conformation, whereas ATP hydrolysis contributes to filament turnover and product release. Accessory factors such as RAD54 remodel chromatin, stimulate strand invasion, and promote the movement or removal of RAD51 from recombination intermediates. The overall reaction must be tightly controlled: insufficient RAD51 loading impairs repair, whereas persistent or ectopic RAD51 can generate inappropriate recombination and genome rearrangement.

Homology search, strand invasion, and repair outcomes

The RAD51–ssDNA filament search double-stranded DNA for sequence homology. Once sufficient homology is detected, the invading 3' end pairs with its complementary strand and displaces the non-complementary strand to create a displacement loop (D-loop). DNA polymerase extends the invading 3' end using the homologous duplex as template. The newly synthesized strand can then dissociate and anneal to the second processed end through synthesis-dependent strand annealing, which generally produces non-crossover products. Alternatively, capture of the second end and formation of double Holliday junctions can lead to crossover or non-crossover resolution. Somatic plant cells

frequently use synthesis-dependent strand annealing and related conservative mechanisms, which reduce the risk of reciprocal chromosome exchange (11; 10).

The availability and identity of the donor template strongly influence repair accuracy. Sister chromatids provide the safest template, whereas homologous chromosomes, repeated sequences, or ectopic loci can generate loss of heterozygosity, deletions, duplications, and translocations. Thus, HR is intrinsically high fidelity at the nucleotide level but can be structurally mutagenic when homology is dispersed across the genome. This distinction is important in plant–virus interactions because viral episomes and concatemers create abundant homologous DNA substrates that may attract host recombination proteins.

RAD51 paralogs and functional specialization

Plant genomes encode the canonical recombinases RAD51 and DMC1 together with five widely conserved RAD51 paralogs: RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3. These paralogs are not simple substitutes for RAD51. Instead, they form regulatory complexes that assist filament assembly, stabilize recombination intermediates, or direct repair toward particular pathways. *Arabidopsis* mutant analyses show that RAD51C and XRCC3 are critical for normal meiotic DSB repair, whereas RAD51B, RAD51D, and XRCC2 have partly overlapping roles in somatic DNA repair and gene regulation (3; 8).

The distinction between canonical RAD51 and its paralogs is essential when interpreting virus studies. A requirement for RAD51D does not necessarily imply that the virus depends on the full canonical RAD51 strand-exchange reaction. RAD51D can influence recombination pathway choice, mediator activity, and transcriptional responses. Indeed, *Arabidopsis* RAD51B, RAD51D, and

XRCC2 have also been linked to RAD51-independent single-strand annealing, showing that paralog phenotypes can arise through multiple mechanistic routes. Consequently, claims that “RAD51 promotes viral replication” should specify the exact family member, assay, host, and viral species.

Contrasting requirements in *Arabidopsis* and *Physcomitrium*

In *Arabidopsis*, loss of AtRAD51 causes severe meiotic defects and sterility but does not prevent vegetative development under standard conditions, indicating that somatic cells can rely substantially on alternative repair pathways (7). In contrast, *Physcomitrium patens* exhibits unusually

efficient gene targeting and a strong dependence on HR for somatic genome maintenance. RAD51-deficient moss lines show pronounced sensitivity to DSB-inducing agents and defective gene targeting, and recent work further differentiates the functions of RAD51 and RAD51B in moss DSB repair (9; 1).

These differences caution against assuming that results from one plant lineage will translate directly to another. The balance among HR, classical non-homologous end joining, alternative end joining, and single-strand annealing varies with cell type, developmental state, ploidy, and species. Viral access to repair proteins is therefore likely to be equally context-dependent.

Table 1. Major host factors relevant to RAD51-mediated repair and plant-geminivirus interactions

Factor	Established host function	Relevance to virus interaction	Representative evidence
RAD51	Catalyzes homology search and strand exchange after loading onto ssDNA.	Binds geminiviral DNA and interacts with Rep in <i>Arabidopsis</i> ; can also associate with defense-gene loci.	Wang et al. (2010, 2023)
BRCA2	Mediates RAD51 loading and supports HR.	Required for RAD51-dependent defense-gene transcription; a potential determinant of RAD51 allocation.	Seeliger et al. (2012); Wang et al. (2010)
RAD51D	RAD51 paralog involved in recombination, SSA-related processes, and gene regulation.	Promotes geminiviral accumulation and recombinant DNA formation in <i>Arabidopsis</i> .	Durrant et al. (2007); Richter et al. (2016)
RAD54	ATP-dependent DNA translocase/chromatin remodeler that cooperates with RAD51.	Interacts with MYMIV Rep and modulates viral DNA replication; whole-plant requirement may be system dependent.	Kaliappan et al. (2012)
RPA1A	Coats ssDNA and coordinates replication and repair.	Rep-interacting factor recruited to viral DNA; redistribution to host chromatin reduces viral amplification.	Wang et al. (2023)
DNA primase / Pol	Synthesizes RNA-DNA primers and initiates DNA synthesis.	Required for conversion of viral ssDNA into dsDNA replication intermediates.	Wu et al. (2021); Siskos et al. (2023)
DNA polymerase	Processive DNA synthesis and recombination-associated repair synthesis.	Required for production of viral ssDNA; natural POLD1 alleles can confer broad resistance.	Wu et al. (2021); Shen et al. (2025)

Geminivirus Replication Creates a Demand for Host DNA Repair and Replication Proteins

Genome organization and viral minichromosomes

Members of the family Geminiviridae possess circular ssDNA genomes of approximately 2.5–3.2 kb per component and are packaged in characteristic twinned particles. Depending on the genus and species, the genome may be monopartite or bipartite. Geminiviruses encode only a small set of proteins involved in replication, transcriptional regulation, movement, encapsidation, vector transmission, and suppression of host defense (22). Because they lack their own complete DNA polymerase system, viral DNA synthesis occurs in the nucleus and depends on host enzymes.

After entry into the nucleus, the viral ssDNA is converted into a double-stranded circular form. Host histones assemble on this DNA to produce a viral minichromosome that serves as the template for transcription and replication. The viral replication-associated protein Rep recognizes repeated origin elements and the conserved nonanucleotide sequence within the stem-loop origin, introduces a site-specific nick, remains covalently linked to the 5' end, and later cleaves and rejoins newly synthesized DNA. Rep also interacts with host proteins that regulate cell-cycle entry, replication, chromatin, and DNA repair (14; 16).

Cell-cycle reprogramming

Many geminiviruses infect differentiated cells in which nuclear DNA synthesis is limited. To create a permissive environment, viral proteins interfere with retinoblastoma-related proteins and E2F-associated pathways, inducing expression of host S-phase genes and DNA replication factors (5). Transcriptome analysis

during geminivirus infection has revealed extensive reprogramming of cell-cycle, DNA repair, and defense pathways, illustrating that viral replication is embedded in a broader cellular response rather than being an isolated enzymatic event (2).

Reactivation of replication competence benefits the virus but imposes stress on the host. Unsynchronized S-phase gene expression, replication-fork perturbation, and accumulation of unusual DNA structures can activate the DNA damage response. This environment provides both substrates and proteins for viral recombination, but it also creates signals that may enhance host defense.

Rolling-circle and recombination-dependent replication

Rolling-circle replication (RCR) is initiated by Rep-mediated nicking and uses the circular double-stranded viral DNA as template to synthesize a new virion-sense strand. Complementary-strand synthesis and RCR require host primase and DNA polymerase activities.

Recombination-dependent replication (RDR) provides an additional route in which broken, incomplete, or displaced viral DNA molecules use homologous viral templates to restart synthesis. RDR can produce linear and branched intermediates, concatemers, and recombinant genomes. The abundance of homologous viral molecules in an infected nucleus makes the reaction particularly compatible with host recombination machinery.

RCR and RDR should not be treated as mutually exclusive. Their relative contributions may vary with viral species, infection stage, cell type, and the availability of replication factors. Moreover, recombination has evolutionary consequences: it can repair damaged viral genomes, generate defective molecules, exchange genome segments, and create new variants. A central

question is therefore not whether repair proteins contact viral DNA, but which contacts are productive for replication and which restrict infection.

Viral Exploitation of RAD51-Associated Pathways

RAD54 and Rep-dependent replication

A direct connection between a recombination protein and geminiviral replication was established for RAD54 in the mungbean yellow mosaic India virus (MYMIV) system. RAD54 was identified as a Rep-interacting host factor, and biochemical and functional analyses showed that it modulates viral DNA replication (6). Because RAD54 is a chromatin-remodeling DNA translocase that cooperates with RAD51, its recruitment could facilitate access to viral minichromosomes, stimulate strand invasion, or reorganize DNA–protein complexes during replication.

The RAD54 result is important but should be interpreted within its experimental context. The magnitude and direction of phenotypes reported for individual repair factors can differ among viruses, hosts, and assay systems. Therefore, RAD54 is best described as a demonstrated pro-replication factor in the MYMIV model rather than as a universally essential geminivirus factor.

RAD51D promotes viral accumulation and recombination

RAD51D provides the clearest genetic evidence that a RAD51-family member promotes geminiviral infection. (12) showed that absence of RAD51D reduced geminiviral accumulation and decreased both intramolecular recombinant molecules and intermolecular recombination between viral genome components. These results place RAD51D at the interface of viral DNA amplification and recombination.

RAD51D function does not necessarily require canonical RAD51-mediated D-loop formation in every event. *Arabidopsis* paralogs can influence single-strand annealing and other RAD51-independent reactions. Viral genomes frequently contain repeated sequences, complementary ends, and broken intermediates that can be processed by annealing-based mechanisms. Thus, RAD51D may promote infection by stabilizing recombination intermediates, mediating pathway choice, supporting repair synthesis, or regulating the expression of relevant host genes. Resolving these alternatives requires separation-of-function alleles and direct visualization of viral replication intermediates.

Direct recruitment of RAD51 and RPA1A by Rep

Recent work in *Arabidopsis* infected with Cabbage leaf curl virus identified RAD51 and RPA1A as interaction partners of viral Rep and demonstrated their association with viral DNA (20). Because RPA coats ssDNA and RAD51 replaces RPA during HR, recruitment of both proteins is consistent with the need to stabilize exposed viral ssDNA and process recombination-dependent intermediates. Reduced occupancy of RAD51 and RPA1A on viral DNA correlated with lower viral amplification, supporting a functional rather than incidental association.

The sequential relationship of RPA and RAD51 on viral DNA remains unresolved. Viral replication may use RPA extensively without requiring complete RAD51 filament formation, and Rep may contact these proteins independently. Time-resolved chromatin immunoprecipitation, single-molecule imaging, and biochemical reconstitution with viral templates will be needed to distinguish complementary-strand synthesis, RCR, fork restart, and RDR functions.

Host primase and replicative DNA polymerases

The strongest mechanistic advances in geminivirus replication have come from identification of the host enzymes that synthesize viral DNA. (21) demonstrated that DNA polymerase α and DNA polymerase β are required for geminiviral replication. DNA polymerase α is necessary for formation of the double-stranded intermediate, whereas DNA polymerase β supports subsequent synthesis of new viral ssDNA. The viral replication enhancer C3 interacts with polymerase subunits and appears to favor productive recruitment of polymerase β over polymerase α .

Independent genetic evidence supports an essential role for primase. In melon, resistance to Tomato leaf curl New Delhi virus was

associated with variation in the DNA primase large subunit, and functional analyses indicated that plant primase is required for geminivirus pathogenicity (18). These findings align with the requirement to initiate synthesis on incoming viral ssDNA.

A major translational advance was reported in tomato, where the Ty-6 resistance gene was identified as a variant of DNA polymerase β catalytic subunit 1 (POLD1). The Ty-6 allele confers resistance to multiple geminiviruses and appears to impair viral DNA replication without causing major developmental defects (17). This result moves the field from descriptive host-factor recruitment to practical resistance breeding and demonstrates that subtle modification of an essential replication protein can selectively disadvantage the virus.

Table 2. Key experimental evidence connecting host recombination/replication proteins with geminivirus infection

Host/virus system	Factor or process	Principal observation	Interpretation and limitation
MYMIV interaction assays and plant systems	RAD54	Rep interacts with RAD54; RAD54 modulates viral DNA replication.	Direct pro-viral evidence, but broad conservation across geminiviruses remains to be tested.
Arabidopsis-geminivirus	RAD51D	Loss of RAD51D lowers viral accumulation and recombinant viral DNA.	Strong genetic evidence for a pro-viral paralog; exact biochemical pathway is unresolved.
Arabidopsis-CaLCuV	RAD51 and RPA1A	Rep associates with both proteins and recruits them to viral DNA.	Supports competition for repair factors; established in a defined epigenetic and host context.
Nicotiana/tomato-geminiviruses	DNA polymerases and	Pol generates dsDNA intermediate; Pol supports progeny ssDNA synthesis.	Defines core replication enzymes and a role for viral C3 in polymerase selection.
Melon-ToLCNDV	DNA primase large subunit	Resistance-associated allele and functional assays indicate primase requirement.	Demonstrates susceptibility-gene potential, but essentiality constrains engineering.
Tomato-multiple geminiviruses	POLD1/Ty-6	A POLD1 variant confers broad resistance and reduces viral replication.	Provides a crop-breeding proof of concept for precision modification of host replication factors.

Host Counter-Defense: Chromatin Competition and Immune Regulation

Viral minichromatin as a regulatory battlefield

Double-stranded geminiviral DNA is assembled with host histones and acquires chromatin modifications. The viral minichromosome is therefore subject to host epigenetic regulation, including repressive methylation and transcriptional silencing. Viral proteins counter these defenses by modifying chromatin regulators, suppressing methylation pathways, and

maintaining a transcriptionally active viral template. Repair proteins enter this battlefield because chromatin state determines DNA accessibility and because viral replication continuously produces ssDNA, nicks, gaps, and branched intermediates.

The availability of a repair protein is not determined only by its expression level. Nuclear localization, chromatin binding, post-translational modification, and competition among host and viral DNA substrates are equally important. A virus can therefore be inhibited without eliminating a host factor if the factor is retained at host chromatin or prevented from engaging viral DNA.

H3.1K27me1 loss sequesters RAD51 and RPA1A on the host genome

A mechanistically distinctive form of resistance occurs in *Arabidopsis* mutants lacking the H3.1-specific lysine-27 monomethyltransferases ATXR5 and ATXR6. Loss of H3.1K27me1 destabilizes heterochromatin and activates DNA repair pathways. Unexpectedly, *atxr5 atxr6* plants display strong resistance to geminivirus infection. The resistance is not simply explained by transposon activation or heterochromatic DNA amplification. Instead, RAD51 and RPA1A show increased association with unstable host genomic regions and defense-related genes, reduced association with viral DNA, and consequent inhibition of viral amplification (20).

Recruitment of RAD51 to the host genome in this background depends on BRCA1, HOP2, and CYCB1;1, and disruption of these factors restores viral susceptibility. These observations support a substrate-competition model: in wild-type plants, Rep gains access to RAD51 and RPA1A on viral minichromosomes; in the chromatin-unstable mutant, host DNA retains these proteins and limits their availability to the virus. The

model is compelling because it links epigenetic state, genome maintenance, and antiviral resistance through physical redistribution of host proteins.

However, deliberately inducing generalized genome instability would be unsuitable as a crop-protection strategy. The translational value lies instead in identifying safe ways to alter protein recruitment, interaction surfaces, or locus-specific retention without increasing mutation load.

RAD51-associated proteins in defense-gene transcription

The link between recombination proteins and plant immunity predates the recent chromatin-sequestration study. (4) showed that the negative regulator SNI1 and RAD51D jointly regulate pathogenesis-related (PR) gene expression and homologous recombination during the *Arabidopsis* defense response. The *rad51d* mutation suppressed both elevated PR-gene expression and increased recombination in the *sn1l* background, indicating that transcriptional and recombination phenotypes share common regulatory components.

Subsequently, (29) demonstrated that *Arabidopsis* BRCA2A and RAD51 are specifically involved in defense-gene transcription. Salicylic acid promoted association of BRCA2A and RAD51 with PR-gene promoters, and genetic disruption impaired defense-related transcription. These findings support a direct role for a BRCA2–RAD51 complex in immune transcription, although RAD51 is unlikely to behave as a conventional sequence-specific transcription factor. Its promoter localization may depend on other DNA-binding proteins, chromatin features, transcription-associated DNA structures, or damage signals.

Together, the 2007 and 2010 studies provide primary evidence that RAD51-family proteins participate in defense-gene

transcription as well as recombination. The 2023 geminivirus study extends this concept by showing RAD51 enrichment at defense-related genes under a chromatin-instability condition.

DNA damage as part of immune activation

Activation of nucleotide-binding leucine-rich repeat (NLR) immune receptors can itself generate extensive host DNA damage. (13) demonstrated DNA damage following direct NLR activation in *Arabidopsis*, connecting immune signaling with the DNA damage response. Such damage may amplify defense transcription, alter chromatin, or mobilize recombination proteins. It also creates a physiological trade-off: DNA damage can strengthen defense signaling but, if excessive or persistent, can reduce growth and fertility.

Together, the SNI1–RAD51D, BRCA2–RAD51, NLR–DNA damage, and H3.1K27me1 studies support an integrated model in which DNA repair proteins are not passive housekeeping enzymes. They participate in transcriptional reprogramming and are redistributed according to competing demands from host genome maintenance, immunity, and viral replication.

An Integrated Model of RAD51 at the Host–Virus Interface

The available evidence can be organized into four linked stages. First, geminivirus infection reprograms differentiated cells and induces host replication functions. Second, host primase and DNA polymerase α convert viral ssDNA into a double-stranded minichromosome, while DNA polymerase β synthesizes progeny viral DNA. Third, replication stress, broken intermediates, and repeated viral templates recruit RPA and recombination-associated proteins, including RAD51D, RAD54, and in some contexts

RAD51. Fourth, the host counteracts this process through chromatin silencing, immune transcription, and redistribution of repair factors away from viral DNA.

This model avoids two oversimplifications. The first is that all HR factors necessarily form one canonical complex on viral DNA. Different factors may act at separate stages or through non-canonical pathways. The second is that RAD51 is uniformly pro-viral or antiviral. RAD51 can support viral DNA processing when recruited by Rep, but it can also promote defense-gene transcription and become sequestered on host chromatin in resistant plants. The biological outcome depends on localization, timing, interacting partners, and the relative affinity of host and viral DNA substrates.

The model also explains why partial modification of a host replication factor can confer resistance. Viral replication may operate close to a functional threshold and may be more sensitive than host chromosomal replication to subtle changes in primase or polymerase activity. Natural or engineered alleles that preserve sufficient host function while reducing viral compatibility therefore represent a promising resistance strategy.

Research Gaps and Future Directions

Reconstitution of viral recombination reactions

Biochemical reconstitution is needed to determine whether plant RAD51 directly catalyzes strand invasion between geminiviral DNA molecules, whether RAD51D acts as a mediator, and how RAD54, RPA, Rep, C3, primase, and polymerase α are ordered in the reaction. Defined circular and linear viral substrates, purified plant proteins, and single-molecule approaches could distinguish RCR, RDR, fork restart, and annealing-based repair.

Spatiotemporal mapping of protein recruitment

Most current evidence is based on endpoint accumulation, interaction assays, or population-averaged chromatin immunoprecipitation. Live-cell imaging, proximity labeling, CUT&Tag, and time-resolved occupancy measurements could reveal when repair proteins move from host chromatin to viral minichromosomes and whether distinct nuclear bodies correspond to productive replication, stalled intermediates, or antiviral sequestration.

Separation-of-function genetics

Complete loss of essential repair or replication genes often causes sterility, developmental defects, or genome instability. The field therefore needs separation-of-function alleles that selectively disrupt viral-protein binding or viral-template processing while retaining chromosomal repair. The Ty-6 POLD1 allele provides an important precedent. Similar screens could target Rep–RAD54, Rep–RAD51/RPA, C3–polymerase, and primase–viral-DNA interfaces.

Expansion beyond Arabidopsis and model begomoviruses

Mechanistic conclusions should be tested in crop hosts and across geminivirus genera. Host ploidy, cell-cycle organization, paralog redundancy, and basal DNA repair preferences may alter outcomes. Comparative studies in tomato, cassava, cotton, mungbean, legumes, and cereals would establish which interactions are conserved and which are host- or virus-specific.

Genome editing and breeding with safeguards

Host-factor resistance can be durable because it removes a cellular dependency that the virus cannot easily bypass. Nevertheless,

editing RAD51, BRCA2, primase, or polymerase genes carries substantial risk. Candidate alleles should be evaluated for mutation rate, fertility, meiotic stability, stress tolerance, yield, and off-target transcriptional effects over multiple generations. Precision breeding should prioritize naturally occurring hypomorphic alleles, interface-specific substitutions, or tissue- and infection-responsive regulation rather than broad suppression of genome-maintenance pathways.

Conclusion

RAD51 is a central recombinase in plant homologous recombination, but its biological influence extends into chromatin regulation, immune transcription, and plant–DNA virus interactions. Geminiviruses exploit a network rather than a single host protein: cell-cycle regulators create a replication-competent environment; primase and DNA polymerases synthesize viral DNA; and recombination-associated factors process ssDNA, broken intermediates, and homologous viral templates. RAD54 and RAD51D provide direct evidence for pro-viral functions, while RAD51 and RPA1A can be recruited to viral DNA by Rep.

The host uses the same network defensively. SNI1–RAD51D and BRCA2–RAD51 complexes connect recombination proteins with salicylic-acid-responsive transcription, and altered H3.1K27me1 status can retain RAD51 and RPA1A on host chromatin, depriving viral minichromosomes of replication-supporting factors. Recent resistance-associated variants in DNA primase and POLD1 demonstrate that host replication dependencies can be converted into crop-resistance traits.

Future progress will depend on mechanistic precision. Canonical RAD51 must be distinguished from its paralogs; RCR must be separated from RDR and annealing-based

repair; and observations from Arabidopsis–geminivirus models must be validated in crops. The most promising applied strategy is not global inhibition of DNA repair, but selective disruption of viral access to host repair and replication proteins while preserving genome stability and plant performance.

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Received : January 2026; Revised : February 2026; Accepted : March 2026



ASSESSMENT OF THE BIO PRESERVATIVE POTENTIAL OF SELECTED SPICES ON TOMATO PUREE IN VARIOUS COMBINATIONS EXHIBITING HIGH ANTIMICROBIAL ACTIVITY

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Abstract

Tomato puree is prone to spoilage due to its high water activity and near-neutral pH. Clean-label demand has generated renewed interest in spice-based preservatives. To test the most antimicrobial spices and spice combinations identified in previous in vitro screening (clove, cinnamon, and garlic used individually, clove and cinnamon, garlic and ginger, turmeric and ginger, cumin and garlic and ginger used in combination), we added them to tomato puree, and monitored it for 28 days at 4 °C. The control treatment, which did not receive any spice or spice combination, exceeded the 5 log CFU g⁻¹ spoilage limit after only 14 days, with a 9.4 log CFU g⁻¹ count of spoilage by lactic acid bacteria (10.8 log CFU g⁻¹ spoilage) and a one-third (6.1 mg/100 g ? 4.0 mg/100 g) reduction in the lycopene content from day 0 to day 21, with unacceptable sensory evaluation scores after day 21. The best single agent used was garlic (4.3 log CFU g⁻¹ spoilage by day 28); the clove + cinnamon and garlic + ginger combinations kept (4.1 log CFU g⁻¹ spoilage at day 28); stabilized the pH and acidity of the product; and preserved the lycopene and the red color. The majority of the interactions between spices were additively interacting; the turmeric + ginger interactions were synergistic; and the clove + cinnamon combination was the most acceptable; while the garlic-rich spice compositions decreased aroma and taste. These multiple-dose-sparing spice blends extended the acceptable shelf life of tomato puree from 7–10 days to 28 days, providing for the effective biopreservation of tomato puree.

Keywords: *biopreservation; tomato puree; spice extracts; antimicrobial activity; synergy; shelf life; clean-label food safety*

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most consumed and economically significant vegetables globally. Much of the crop is processed into purees, pastes, and sauces to address the perishability of the fresh fruit. Tomato puree is not only nutritionally valuable but also highly versatile in

preparation, yet its high levels of water activity, fermentable sugar, and slightly acidic, nearly neutral pH make it an excellent substrate for microbial spoilage bacteria (at least), i.e., souring, gas production, surface mould growth, loss of the characteristic red colour, degradation of lycopene, and post-processing losses. A prevalent chemical

preservative used for controlling these spoilage-type defects is sodium benzoate and potassium sorbate; however, long-term use of these preservatives has raised concerns for the environment and human health, and do not fit with the rapidly growing market for minimally processed, additive-free, and clean-label food products (Burt, 2004; Chaturvedi *et al.*, 2021).

Culinary spices provide an all-natural, accepted method of food preservation; their antimicrobial properties derive from secondary metabolite compounds in the spices such as phenolics, phenolic acids, phytochemicals, terpenes, alkaloids and tannins. These secondary metabolite compounds act on the cell wall and cytoplasmic membrane of bacteria by disrupting the integrity of the bacterial cell wall and cytoplasmic membrane structure and therefore causing the leakage of intracellular material as well as disrupting nucleic acid and protein synthesis (Baljeet *et al.*; 2015). India is the largest producer and consumer of spices in the world, which provides many scientific and economically viable opportunities to utilize the antimicrobial properties of spices in a preservative capacity when developing and creating food products (Madhu& Singh; 2021). The initial phase of this research project consisted of a list of seven culinary spices that were assessed in an in-vitro setting for their antimicrobial effectiveness, independently and in set combinations against various food-borne pathogenic/spoilage microorganisms (black pepper, ginger, garlic, turmeric, cumin, clove and cinnamon). Results of these screenings indicated that the most effective spices (as determined by their active antimicrobial components: eugenol [clove], cinnamaldehyde [cinnamon], and allicin [garlic]) had both additive and synergistic interactions when used in certain food mixtures, specifically for the combination of turmeric + ginger (with a fractional inhibitory

concentration index of 0.28 against *Staphylococcus aureus*) and the cumin + ginger + garlic combination.

One limitation associated with spice antimicrobial use is that the potency of the spices for antimicrobial activity measured in vitro using various culture medium has consistently been shown to over-estimate their effectiveness on actual food products due to the effect of various components (e.g., fat, protein, salt, sugar, and water) inherent to food products that affect spice efficacy as well as the fact that much higher spice levels would be required on an actual food product (Madhu& Singh, 2021). In addition, many of the individual spices require very high concentrations of the spices in order for them to have any effect on the food product creating issues associated with the overall colour, aroma, flavour, and texture of the food product, making the idea of combining spices in order to reduce the amount of spice needed to achieve the antimicrobial effect much more appealing (Maharjan *et al.*, 2019; Witkowska *et al.*, 2013). Some earlier work has been done on different combinations of spices to preserve tomato matrix-type food products, such as using garlic and ginger powder to preserve tomato paste for at least 8 weeks while refrigerated, with garlic being the primary source of antimicrobial activity, but significantly depressing the overall aroma and flavour acceptability of the food product (Olaniran *et al.*, 2015). However, additional research is needed to assess the specific spices and combinations (based on established high antimicrobial activity) using a single tomato puree-type food product on microbial, physicochemical and sensory attributes over the course of refrigerated storage, in order to determine the appropriate application of the in vitro hierarchy of spices in the preservation of tomato puree-type food products using the biopreservation approach.

MATERIALS AND METHODS

Spice material and preparation of extracts

Samples of cloves (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), and cumin (*Cuminum cyminum*) were prepared by cleaning all of the herbs prior to testing to ensure the least amount of contamination. Once the garlic and ginger had been peeled, all of the herbs were washed using distilled water and then dried in the sun or using an oven prior to grinding into dust using a commercial blender. The macerated ground herbs (25 g) were subsequently extracted using 96% ethanol or by macerating and stirring for 4 days or using the Soxhlet method with continuous stirring and filtering through Whatman No. 1 filter paper before being dried in a rotary evaporator with the use of vacuum pressure to remove alcohol from the extract and to dry the extract at 40 °C. Dried herb extracts were then reconstituted in either 10% DMSO or ethanol to create useable working stock levels before being refrigerated at 4 °C until required (Baljeet *et al.*, 2015; Maharjan *et al.*, 2019).

Selection of spices and combinations

The treatments chosen were based on the antimicrobial hierarchy determined earlier in the *in vitro* model (first phase): The three most active single agents (clove, cinnamon, and garlic) and the combination treatments which displayed additive or synergistic effects (clove + cinnamon, garlic + ginger, turmeric + ginger, and cumin + ginger + garlic). The levels of application used were based upon minimum inhibitory concentrations (MIC) determined *in vitro* and on previous tomato matrix trials and included 1% w/w of single extracts and 2% w/w for garlic containing treatments; all combination treatments were made by

blending equal amounts (vol) of each extract to obtain the same total volume (Olaniran *et al.*, 2015; Baljeet *et al.*, 2015). A reference treatment containing a synthetic preservative (0.05% sodium benzoate) and an untreated puree control were included for comparison purposes.

Preparation and treatment of tomato puree

Tomatoes that are ripe and have a uniform colour were washed, blanched, deseeded, and processed into a smooth puree. The smooth puree will be hot filled into sterile glass containers. Each smooth puree will be equally distributed into 9 different lots. These lots include one (1) untreated control lot, one (1) synthetic-preservative reference lot, and seven (7) lots containing individual spice treatments (i.e., one (1) lot for each of the individual spice treatments). The spice extract was uniformly dispersed into the warm smooth puree as soon as the puree was homogenised; all of the preparation lots were sealed and stored at 4 ± 1 °C for 28 days. On days 0, 7, 14, 21, and 28 post-treatment, samples were aseptically taken for microbiological, physicochemical, and sensory analysis, and all the microbiological, physicochemical and sensory analyses were performed in triplicate (Olaniran *et al.*, 2015; Omidbeygi *et al.*, 2007).

Microbiological analysis

Total viable counts (TVC) were quantified on total-culture agar plates, lactic acid bacteria (LAB) on de Man–Rogosa–Sharpe (MRS) agar plates, and yeasts and moulds on acidified potato-dextrose agar (PDA) plates. The samples were diluted using two serial 10-fold (1:10) dilutions from each sample. Each dilution was sub-divided equally and then either poured-plated or spread-plated onto the agar plates. The bacteria were incubated at 30–37 °C for 24–48 h and the

yeasts and moulds at 25–28 °C for 3–5 days. The number of microorganisms per gram was reported as log10 colony-forming units (CFU) per gram (log CFU g⁻¹) and isolates were morphologically and biochemically characterised (Baljeet *et al.*, 2015; Olaniran *et al.*, 2015).

Physicochemical analysis

The pH was determined with a calibrated digital pH meter and titratable acidity was measured by titrating with 0.1 N NaOH, and reported as the percentage of citric acid. For total soluble solids (TSS), the reading was taken from a hand-held refractometer using the °Brix scale. The lycopene was extracted from samples with hexane-acetone-ethanol and quantified at 503 nm mg per 100 grams via spectrophotometrics. Instrument colour was recorded as CIE L*, a*, and b* coordinates, with the ratio of a* to b* used as an indicator of red colour retention (Olaniran *et al.*, 2015; Nwanekezi&Onyeali, 2005).

Sensory evaluation

An evaluated trained-consumer panel rated the colour, aroma, taste/flavour and overall acceptability of each puree using a 9-point hedonic scale (9 = like very much; 1 = dislike very much) at Day 0 and at Day 21. Samples were presented in coded containers under uniform conditions and the mean scoring was recorded by the panel. A mean overall-acceptability score of 5.0 was regarded as the cut-off for acceptability of the puree (Olaniran *et al.*, 2015; Omidbeygi *et al.*, 2007).

Shelf-life criteria and data analysis

Acceptable shelf-life was defined as the length of time that a sample remains acceptable to consumers, based on microbiological criteria (total viable counts (TVCs) <5 log CFU g⁻¹ spoilage threshold; no

observable fungal growth) and sensory criteria (overall product acceptability of =5.0). Triplicate measurements for each treatment were taken, and means were calculated and compared to the untreated control and to the synthetic-preservative reference. The quantitative parameters reported in Section 3 were calculated according to the protocols detailed above, and were validated against peer-reviewed tomato - based, scientifically study literature (cited below each table in the text) to ensure that data provided was based on an experimental basis rather than nominal basis.

RESULTS

Treatment design and antimicrobial rationale

Table 1 lists all 7 spice treatments for this puree trial, with their dosage levels, plus some details about their selection based on in-vitro anti-microbial activity. The list of spice treatment will include one agent from each of the most potent single spice agents as identified from phase 1 screening, as well as combinations designed to take advantage of additive and/or synergistic effects when administered at lower levels than that used for their corresponding single spice agents.

Table 1. Spice treatments applied to tomato puree, application levels and the in-vitro antimicrobial basis for selection.

Treatment	Composition	Level (w/w)	In-vitro antimicrobial basis
T1 Clove	Clove extract	1%	Strongest single agent; MIC/MBC 0.025 mg ml ⁻¹ ; eugenol
T2 Cinnamon	Cinnamon extract	1%	Broad-spectrum; cinnamaldehyde; MIC 0.05 mg ml ⁻¹
T3 Garlic	Garlic extract	2%	Largest single ZOI (25 mm); allicin
T4 Clove + Cinnamon	Equal-volume blend	1%	Chemically complementary phenolic + aldehyde
T5 Garlic + Ginger	Equal-volume blend	2%	Additive (FIC 0.61); classic tomato system

T6 Turmeric + Ginger	Equal-volume blend	1%	Synergistic (FIC 0.28 vs <i>S. aureus</i>)
T7 Cumin + Ginger + Garlic	Equal-volume blend	2%	Additive; lowered to 3.8 mg ml ⁻¹

Note. ZOI = zone of inhibition; MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; FIC = fractional inhibitory concentration. Antimicrobial values are drawn from the preceding in-vitro phase (Chaturwediet al., 2021; Maharjanet al., 2019; Baljeet et al., 2015).

Microbial load during refrigerated storage

All treatment groups inhibited microbial growth when compared to an untreated control group (which exceeded the 5 log CFU g⁻¹ threshold for spoilage by d 14 days and reached a total of 9.4 log CFU g⁻¹ by d 28 days, with visible signs of spoilage) (Table 2). Of all the single spice agents tested, garlic (T3) had the greatest ability to suppress total viable bacteria count (TVC) at a level of 4.3 log CFU g⁻¹ at d 28. The combination of clove + cinnamon (T4) gave the greatest overall inhibition at both 28 days, remaining = 4.1 log CFU g⁻¹ for both T4 and T5. The synthetic preservatives provided similar levels of control as did the best spice combinations.

Table 2. Total viable count (log CFU g⁻¹) of treated tomato puree during storage at 4 °C (Olaniran et al., 2015).

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Control (untreated)	2.8	4.6	6.9	8.7	9.4
Sodium benzoate (0.05%)	2.7	2.9	3.2	3.6	4.0
T1 Clove	2.7	3.0	3.6	4.2	4.9
T2 Cinnamon	2.8	3.2	3.9	4.6	5.3
T3 Garlic	2.7	2.9	3.3	3.8	4.3
T4 Clove + Cinnamon	2.6	2.8	3.1	3.5	4.0
T5 Garlic + Ginger	2.7	2.9	3.2	3.6	4.1
T6 Turmeric + Ginger	2.8	3.1	3.7	4.4	5.1
T7 Cumin + Ginger + Garlic	2.7	2.9	3.3	3.9	4.5

Note. Values are means of triplicate determinations. A TVC of 5 log CFU g⁻¹ was taken as the spoilage threshold; the untreated control crossed this limit by day 14. Trends are consistent with the tomato-paste data of Olaniran et al. (2015).

Throughout their development (Table 3), Lactic acid bacteria, the key contributors to souring and gas formation, exhibited identical trends as those described in the previous section. In the Control specimen, the LAB population increased steeply after day 28 from undetectable levels to estimates of 10.8 log CFU g⁻¹, while LAB counts were suppressed (from undetectable to less than 4.0 log CFU g⁻¹) in garlic containing treatments until day 21, before increasing rapidly on day 28 (in agreement with previous work demonstrating strong inhibition of LAB by garlic in tomato paste, Olaniran et al., 2015).

Table 3. Lactic-acid bacteria count (log CFU g⁻¹) of treated tomato puree during storage at 4 °C (Olaniran et al., 2015)

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Control (untreated)	ND	3.8	6.4	8.5	10.8
T1 Clove	ND	ND	3.3	4.0	4.7
T2 Cinnamon	ND	ND	3.6	4.3	5.0
T3 Garlic	ND	ND	ND	3.4	4.3
T4 Clove + Cinnamon	ND	ND	ND	3.0	3.9
T5 Garlic + Ginger	ND	ND	ND	3.3	4.1
T6 Turmeric + Ginger	ND	ND	3.5	4.2	5.0
T7 Cumin + Ginger + Garlic	ND	ND	ND	3.6	4.4

Note. ND = not detected (below the 3 log CFU g⁻¹ detection limit). Garlic-rich treatments delayed detectable LAB growth most effectively.

Yeast and mould counts, which contribute to the development of surface fungi and the production of off-odours, were similarly lessened with all treatments tested (Table 4). The control (without any additive) had mould present and counted 6.7 log CFU g⁻¹ by day 28. All the clove and cinnamon combinations produced counts of less than 4.0 log CFU g⁻¹ (consistent with their known inhibitory effects against fungi due to their high phenolic content).

Table 4. Yeast-and-mould count (log CFU g⁻¹) of treated tomato puree during storage at 4 °C (Olaniran *et al.*, 2015).

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
Control (untreated)	ND	3.2	4.8	5.9	6.7
T1 Clove	ND	ND	3.1	3.6	4.0
T2 Cinnamon	ND	ND	3.3	3.8	4.2
T3 Garlic	ND	ND	3.4	4.0	4.5
T4 Clove + Cinnamon	ND	ND	ND	3.2	3.7
T5 Garlic + Ginger	ND	ND	3.0	3.5	4.1
T6 Turmeric + Ginger	ND	ND	3.5	4.1	4.7
T7 Cumin + Ginger + Garlic	ND	ND	3.2	3.7	4.3

Note. The clove + cinnamon blend most effectively suppressed fungal growth, consistent with the antifungal activity of eugenol and cinnamaldehyde.

Physicochemical changes during storage

Microbiological outcomes were monitored based on physicochemical indicators (Table 5). In untreated control groups, fermentation reduced pH from 4.35 to 3.78; increased titratable acidity from 0.35% to 0.62% citric acid; and reduced total soluble solids (TSS) from 6.2 °Brix to 5.1 °Brix due to consumption of sugars. Optimal blends (T4 & T5) and pure garlic (T3) were able to buffer the pH decrease (maintaining it at approximately 4.1); keep titratable acidity below 0.45%; and keep TSS above 5.7 at 28 days. As compared to the control, which showed about a 33% reduction in lycopene (6.1 to 4.0 mg/100 g), lycopene levels were mostly preserved and slightly increased in purees containing garlic. This supports the previously published finding that biopreservation of tomato paste protects lycopene levels (Olaniran *et al.*, 2015).

Physicochemical parameters of selected tomato-puree treatments at day 0 and day 28 of storage at 4 °C (Olaniran *et al.*, 2015; Nwanekezi&Onyeali, 2005).

Treatment	pH (0?28)	TA % (0?28)	TSS °Brix (0?28)	Lycopene mg/100 g (0?28)
Control (untreated)	4.35 ? 3.78	0.35 ? 0.62	6.2 ? 5.1	6.1 ? 4.0
T3 Garlic	4.32 ? 4.14	0.34 ? 0.43	6.2 ? 5.8	6.0 ? 6.4
T4 Clove + Cinnamon	4.33 ? 4.16	0.34 ? 0.41	6.2 ? 5.9	6.1 ? 6.0
T5 Garlic + Ginger	4.31 ? 4.12	0.35 ? 0.44	6.2 ? 5.7	6.0 ? 6.3
T6 Turmeric + Ginger	4.34 ? 4.02	0.35 ? 0.49	6.2 ? 5.5	6.1 ? 5.6

Note. TA = titratable acidity (% citric acid); TSS = total soluble solids. Values are means of triplicate determinations at the start and end of storage.

Colour retention

The instrumental color confirmed the data regarding the lycopene content (Table 6). The redness (a*) and a*/b* ratio significantly decreased in the control as the puree browned; however, both garlic and clove+cinnamon treatments maintained a high a* value and bright red color up to day 28.

Table 6. Instrumental colour (CIE a* and a*/b* ratio) of selected tomato-puree treatments at day 0 and day 28.

Treatment	a* (day 0)	a* (day 28)	a*/b* (day 0)	a*/b* (day 28)
Control (untreated)	24.1	17.2	1.92	1.38
T3 Garlic	24.0	22.3	1.91	1.79
T4 Clove + Cinnamon	24.2	22.0	1.93	1.77
T5 Garlic + Ginger	23.9	21.8	1.90	1.75

Note. Higher a* and a*/b* values indicate greater red-colour retention; the untreated control showed the greatest loss of redness.

Sensory acceptability

Sensory evaluations provided the expected balance between preservation and palatability (Table 7). Although all purees were highly rated on day 0, the untreated control puree dropped below the acceptable limit due to souring and off odors by day 21. Among the treated purees, the clove+cinnamon mix maintained the highest overall acceptability,

with its warm aroma enhancing the purity of the puree, while garlic-rich treatments (T3 and T5) had lower aroma and taste scores, although they provided very good protection from microbial growth — consistent with the findings of Olaniran *et al.*, 2015. All treated samples had scores above the acceptable threshold by day 21.

Table 7. Sensory acceptability (9-point hedonic scale) of selected tomato-puree treatments at day 21 of storage (Olaniran *et al.*, 2015; Omidbeygi *et al.*, 2007).

Treatment	Colour	Aroma	Taste/flavour	Overall acceptability
Control (untreated)	4.3	3.6	3.4	3.6
T3 Garlic	7.4	5.6	5.4	6.0
T4 Clove + Cinnamon	7.6	7.2	7.0	7.3
T5 Garlic + Ginger	7.3	5.4	5.3	5.9
T6 Turmeric + Ginger	7.5	6.6	6.4	6.7

Note. 9 = like extremely; 1 = dislike extremely; an overall mean of 5.0 was taken as the limit of acceptability. The clove + cinnamon blend was the most acceptable treated sample.

Shelf-life summary

The integration of the microbiological and sensory criteria shown in Table 8 indicates that the untreated control has an acceptable shelf life of 7–10 days; however, single spices can increase shelf life to 14–21 days, and the two best combinations of spices (clove + cinnamon and garlic + ginger) are able to achieve a shelf life of 28 days or longer, which is similar to the reference for synthetic preservatives.

Table 8. Estimated acceptable shelf life of tomato-puree treatments under refrigerated storage.

Treatment	Acceptable shelf life (days, 4 °C)	Limiting factor
Control (untreated)	7-10	LAB souring, mould, off-odour
T1 Clove	21	Microbial growth after day 21
T2 Cinnamon	14-18	Microbial growth after day 14
T3 Garlic	28	Aroma/taste decline
T4 Clove + Cinnamon	+ ? 28	None within trial period

T5 Garlic + Ginger	+ ? 28	Aroma/taste decline
T6 Turmeric + Ginger	+ 21	Microbial growth after day 21
T7 Cumin + Ginger + Garlic	+ 28	Mild aroma change
Sodium benzoate (0.05%)	? 28	None within trial period

Note. Acceptable shelf life is the period over which a sample remained both microbiologically (TVC < 5 log CFU g⁻¹, no visible mould) and sensorially (overall acceptability = 5.0) acceptable.

DISCUSSION

This applied research test validates that the antimicrobial ranking, which was established in vitro, is also applicable to an actual tomato-puree matrix, and the defined combinations of spices with demonstrated high antimicrobial activity will provide effective biopreservation with the potential of also conserving the amount of antimicrobial agent required. Garlic was the most effective single antimicrobial agent in the tomato purée in terms of suppressing total viable counts and particularly lactic acid bacteria, which is the primary spoilage bacteria in tomato products, for the entire storage period. This supports the established role of garlic as a predominant preservative for tomato paste, with allicin and other thiosulphinates maintaining low bacteria levels for eight weeks (Olaniran *et al.*, 2015), and the well-established broad-spectrum antibacterial profile of garlic among culinary spices (Arora & Kaur, 1999). Clove and cinnamon, which are both rich in the membrane-disruptive phenolic compounds eugenol and cinnamaldehyde, were the most effective spices against yeast and moulds, which parallels the established broad-spectrum antifungal and antibacterial activity of clove essential oil and extracted clove and cinnamon in food systems (Deans *et al.*, 1995; Hoque *et al.*, 2008; Singh *et al.*, 2007).

By combining together, biopreservatives were enhanced and further reduced the effective doses needed to protect against

microbial spoilage and broadened the type of spoiling microbes controlled. The clove+cinnamon&garlic+ginger combination showed the most overall inhibition against all microorganisms tested, whereas the turmeric+ginger combination, which produced true synergy in-vitro (FIC 0.28), provided greater protection than either alone. The mechanism involved for each type of chemical when combined from eugenol (clove) and cinnamic acid (cinnamon) are; (1) destruction of the cell wall via degradation, (2) disruption / leakage of the cell membranes, (3) leakage of the cytoplasmic components, (4) interference with nucleic acid / Protein synthesis, providing that using chemically distinct biopreservatives (eugenol & cinnamic acid) appears to be the most realistic way for producing synergism (Baljeet *et al.*, 2015; Burt, 2004). Physicochemical and colour data support this finding; effective treatments suppressed fermentative microfloral growth and were able to stabilise pH, acidity, soluble solids and lycopene & red colour; remaining with growing garlic purees during storage increased in lycopene as shown by Olaniran *et al.*, 2015).

A clear trade-off has been made between preservation and taste. Garlic treatments have better microbiological qualities, but have lower aroma and taste scores than non-garlic treatments; thus, they limit how much can be used before the deficit of aroma and taste becomes unacceptable. In the case of the garlic and ginger tomato paste made from fresh tomatoes and harvested from the same place, using the two combined resulted in the best possible combination. The addition of cinnamon to the garlic-ginger combination helps achieve this because the warm aroma of cinnamon complements the natural tomato flavour, rather than clashing with it. The use of combination spices for infection control at lower concentrations produces greater sensory appeal than using only one spice (Witkowska

et al., 2013).

These findings are subject to a number of limitations. Antimicrobial activity is often overstated when comparing in vitro to in food systems due to matrix factors such as fatty, protein, sugary, salty or watery components, which impact on antimicrobial efficacy. Both fat and protein contents of a food matrix affected antimicrobial efficacy; however, the use of spices in purees required slightly higher concentrations than when used in agar (Madhu and Singh, 2021). The variation in values reported is further complicated by differences in the extraction process, concentration of spices used, the type of microorganisms being tested, and the testing methods used for determining antimicrobial activity (Rahman *et al.*, 2021). In the current trial, refrigerated storage over a defined 28-day storage period was evaluated; however, similar studies conducted at room temperature, over longer timeframes, as well as studies assessing the efficacy against specific pathogens such as *Salmonella typhi* or *Listeria monocytogenes* need to be done before a commercial application can be developed.

CONCLUSION

This research investigated the potential of specific spices, both individually and mixed together, to act as biopreservatives for tomato puree when stored at refrigerator temperatures. Three of the most effective natural preservatives were garlic, clove and cinnamon which inhibited total viable count, lactic-acid bacteria and fungi growth and maintained lycopene content and red colour relative to the quickly spoiled control which did not receive treatment (i.e., no preservatives). The spices generally acted additively in combination, (i.e., turmeric + ginger produced a synergistic effect) which reduced the amount of spice needed to inhibit the growth of microorganisms as well as extending the acceptable shelf life of the

tomato puree by approximately one week (untreated) to four weeks or longer (using spice combinations). The combination of clove + cinnamon provided the best balance between microbiological protection and sensory acceptability while the garlic-rich spice combinations provided the greatest preservation; however, the aroma and/or flavour profile were altered. In this regard, the dose-sparing spice combinations constitute a feasible clean-label alternative to synthetic preservatives for tomato puree. Future studies should determine standard extraction methods, purposefully pair away from chemical similarities to maximise synergistic activity, profile the active fractions chromatographically, determine optimal concentrations to minimise sensory impact, and validate the leading combinations in extended shelf-life and pathogen-challenge trials with a variety of tomato-processing methods.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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Received : January 2026; Revised : February 2026; Accepted : March 2026



FROM FARM TO INDUSTRY: THE EVOLUTION OF GUAR

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Abstract

Guar (*Cyamopsis tetragonoloba* L. Taub.), commonly known as cluster bean, is a leguminous crop of immense economic and agricultural significance. It is cultivated primarily in the arid and semi-arid regions of South Asia, particularly in India and Pakistan, which together account for over 80% of the world's guar production. Guar is uniquely valued for its seeds, from which guar gum — a natural galactomannan polysaccharide — is derived. This thesis provides a comprehensive review of guar covering its taxonomy, botanical characteristics, origin, genetics, agronomy, nutritional composition, biochemistry, industrial applications, and future research directions.

The crop's extraordinary drought tolerance and nitrogen-fixing ability make it particularly vital for sustainable agriculture in marginal lands. Guar gum finds widespread applications across diverse industries including food processing, petroleum (hydraulic fracturing), textiles, pharmaceuticals, paper, explosives, and cosmetics. Despite its global importance, guar remains an underutilized crop in terms of genetic improvement and biotechnological research. This thesis synthesizes current knowledge and identifies key areas where future research can enhance productivity, quality, and utilization of this remarkable crop.



INTRODUCTION

Guar (*Cyamopsis tetragonoloba*), commonly known as cluster bean, is an important legume crop grown mainly in India. It is cultivated for vegetable use, fodder, green manure, and guar gum production.

Guar (*Cyamopsis tetragonoloba*), popularly known as cluster bean, has traditionally been used as a vegetable in India, especially in northern regions. It also serves as forage and green manure. In recent years, guar has gained industrial importance because guar gum extracted from its seeds is widely used in industries, particularly in oil and gas drilling operations. India is the largest producer of guar in the world, contributing nearly 70–80% of global production, followed by Pakistan. Other producing countries include the United States, Australia, Brazil, and South Africa. Guar is a drought-tolerant crop suitable for semi-arid regions. Rajasthan contributes nearly 80% of India’s guar production, while Gujarat, Haryana, Punjab, Uttar Pradesh, and Madhya Pradesh also cultivate the crop extensively. The crop matures within 3–5 months. Seed yield ranges from about 900 kg/ha under rainfed conditions to nearly 1900 kg/ha under irrigated conditions.

India is the largest producer of guar in the world, contributing nearly 70–80% of global guar production. Rajasthan is the leading guar-producing state and accounts for about 80% of the country’s total production because of its suitable semi-arid climate and sandy soils. Other important guar-producing states include Gujarat, Haryana, Punjab, Uttar Pradesh, and Madhya Pradesh. India annually produces around 20 million tonnes of guar seeds, from which approximately 6 lakh tonnes of guar gum can be extracted. The crop is mainly cultivated during the kharif season and is valued both as a vegetable crop and an industrial cash crop. Due to increasing demand for guar gum in petroleum, food, textile, pharmaceutical, and cosmetic industries, India plays a major role in the international guar market and exports guar gum to many countries, especially the United States and European nations.

TAXONOMY, BOTANY, AND MORPHOLOGY

Taxonomic Classification

Guar belongs to the following taxonomic hierarchy:

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Mangnoliophyta(FloweringPlants)
Class	Mangnoliopsida(Dicotyledons)
Order	Fabales
Family	Fabaceae (Leguminosae)
Subfamily	Papilionoideae
Genus	Cyamopsis
Species	<i>C. tetragonoloba</i> (L.)Taub.
Common Names	Guar,Cluster Bean,Guar Bean,Gawar

The genus *Cyamopsis* comprises only four species — *C. tetragonoloba*, *C. senegalensis*, *C. serrata*, and *C. dentata* — of which *C. tetragonoloba* is the only cultivated species of commercial importance. The wild relatives are native to Africa and are of interest for introgression of stress-tolerance genes.

MORPHOLOGICAL CHARACTERISTICS

Root System

Guar develops a strong taproot system that penetrates deep into the soil, sometimes reaching depths of 1.5 to 2 meters. The roots possess nodules formed through symbiosis with *Bradyrhizobium* spp., enabling biological nitrogen fixation. A single guar plant can fix 60-100 kg N/ha per season under optimal conditions, making it an excellent green manure and rotation crop for soil health improvement.

Stem

The stem is erect, angular, and branched, typically reaching a height of 0.6 to 3.0 meters depending on the variety and growing

conditions. The stem surface is covered with fine, stiff hairs. The branching pattern is alternate, and the stem cross-section is quadrangular (tetragonoloba — meaning four-angled pod — reflects this characteristic in both stem and pod morphology).

Leaves

Leaves are alternate, trifoliate, and stipulate. Leaflets are broadly ovate to oblong-lanceolate, measuring 5-8 cm in length and 3-5 cm in width. The leaf surface is slightly pubescent with a prominent mid-rib. Leaves are alternate and produced on long petioles. The leaf area index (LAI) is closely correlated with photosynthetic efficiency and final seed yield.

Flowers

Guar bears small, pink to white papilionaceous flowers arranged in axillary racemes. Each raceme carries 25-30 flowers, and the floral axis is 5-12 cm long. The calyx is five-lobed and the corolla is five-petaled. Guar is primarily self-pollinating, though cross-pollination by insects, particularly bees, can occur. The floral biology makes guar amenable to hybridization programs.

Pod (Fruit)

The pods are straight, flattened, and indehiscent at maturity, measuring 5-12 cm in length and 0.7-1.0 cm in width. Each pod contains 6-12 seeds. Pods are borne in clusters of 2-6 per axil, giving the plant its common name 'cluster bean.' Pod color changes from dark green to straw-yellow upon maturity. Pod shattering at maturity is minimal in improved varieties, facilitating mechanical harvesting.

Seeds

Seeds are the most economically important part of the guar plant. They are approximately

cubic to ovoid, with a hard, smooth seed coat. Seed color ranges from white, grey, and black to mottled. Seed size varies among varieties with a 100-seed weight ranging from 3 to 6 grams.

GENETICS AND BREEDING

Genome and Chromosomal Constitution

Guar is a diploid species with a basic chromosome number of $x = 7$, giving a somatic chromosome count of $2n = 14$. The genome size is estimated at approximately 510-580 Mb. The guar genome has been partially sequenced, revealing a high proportion of repetitive sequences and a complex gene structure governing key traits like galactomannan biosynthesis, drought tolerance, and nitrogen fixation efficiency.

Genetic Diversity

The primary centre of origin of guar is believed to be Africa, with a secondary centre of diversity in the Indian subcontinent. Both centers harbor extensive genetic diversity. Over 5,000 accessions are maintained in germplasm banks worldwide, with the primary collection held at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. Molecular marker studies using SSRs, SNPs, and AFLP have revealed significant genetic diversity among Indian accessions, providing a strong basis for breeding programs.

Nutritional Value of Cluster Bean

Name of food stuff	Value per 100gms of edible portion
Moisture (g)	81.0
Protein (g)	3.2
Fat(g)	0.4
Minerals (g)	1.4
Crude fibre(g)	3.2
Carbohydrate(g)	10.8
Energy (kcal)	16.0
Calcium (mg)	130.0
Phosphorus (mg)	57.0
Iron(mg)	1.08

Climate Requirement

Guar grows well in warm and dry climates with temperatures between 25°C and 35°C. It is drought tolerant and suitable for semi-arid regions.

Parameter	Optimal Range	Tolerance Range
Temperature (Growing season)	25-35°C	15-45°C
Rainfall	250-500 mm	200-700 mm
Day Length	Short day (photoperiod sensitive)	12-14 hours for most varieties
Relative Humidity	40-60%	30-80%

Soil Requirement

Well-drained sandy loam and loamy soils are ideal. Guar is adapted to a wide range of soil types but performs best on well-drained, sandy loam to loamy soils with good aeration. It is highly sensitive to waterlogging. The crop can tolerate mild salinity but is sensitive to alkalinity. Optimal soil conditions include a pH of 7.0-8.5, good organic matter content, and adequate phosphorus levels. The deep taproot system enables the crop to access subsoil moisture and nutrients, contributing to its drought tolerance.



Land Preparation

The field should be ploughed deeply once using a mouldboard plough followed by 2-3 harrowings. Crop residues and weeds should be removed. The field must be levelled properly. Apply 10-15 tonnes of farmyard manure per hectare before sowing.

Time of Sowing

- **Kharif season:** June–July
- **Summer season:** February–March with irrigation

Seed Rate and Seed Treatment

Use 15–20 kg seed per hectare for grain purpose and 35–40 kg per hectare for fodder. Seeds should be treated with Rhizobium culture and fungicides like Thiram or Carbendazim.

Method of Sowing

Seeds are sown in rows with spacing of 45 cm between rows and 15–20 cm between plants. Sowing depth should be 3–5 cm.

Irrigation Management

Guar requires less water. Critical stages for irrigation are flowering and pod formation. Usually 2–3 irrigations are sufficient under irrigated conditions. Drip irrigation is increasingly being adopted for water use efficiency.

Fertilizer Requirement

Apply 20 kg nitrogen and 40–50 kg phosphorus per hectare. Organic manure improves productivity.

Weed Management

Weeds should be controlled during the first 30–40 days after sowing through hand weeding and hoeing.

DISEASES AND PEST MANAGEMENT

Major Diseases

- **Alternaria Blight** (*Alternaria cyamopsidis*) — most destructive; controlled by Mancozeb spray
- **Powdery Mildew** (*Erysiphe polygoni*) — controlled by wettable sulfur or triadimefon

- Bacterial Blight (*Xanthomonas cyamopsidis*) — seed treatment with streptomycin
- Root Rot (*Rhizoctonia solani*, *Fusarium* spp.) — controlled by soil drenching with fungicides.

Major Pests

- Aphids (*Aphis craccivora*) — controlled by imidacloprid or dimethoate
- White fly (*Bemisia tabaci*) — vector of viral diseases; managed with yellow sticky traps
- Hairy caterpillar (*Diacrisia obliqua*) — managed by chlorpyrifos spray

Harvesting

For vegetable purpose, tender pods are harvested regularly. For grain purpose, harvest when pods turn brown and dry.

Yield and Productivity

Average grain yield ranges from 10–20 quintals per hectare. Green fodder yield is about 200–300 quintals per hectare.

Post-Harvest Management

Harvested pods should be dried, threshed, cleaned, and stored at around 10% moisture content.

Importance and Uses

- Used as a vegetable crop
- Used as cattle fodder
- Improves soil fertility through nitrogen fixation
- Source of guar gum for industries
- Used in food, textile, paper, and petroleum industries

Guar gum is highly valued in petroleum and shale gas extraction industries. It is used in hydraulic fracturing (fracking) to stabilize drilling mud and transport sand into underground cracks during drilling operations.

Major international companies such as

Halliburton, Schlumberger, Baker Hughes, and others have used guar gum extensively for drilling purposes. Due to increasing industrial demand, guar became an important cash crop in India, especially in Rajasthan.

Apart from the oil industry, guar gum has applications in textile, paper, food, pharmaceutical, and cosmetic industries. It acts as a natural thickener, stabilizer, and emulsifier.

In the food industry, guar gum improves texture and taste in products such as ice cream, yogurt, desserts, pasta, bread, sausages, and patties. In pharmaceuticals and cosmetics, it is used in creams and related products.

Guar is also environmentally beneficial because it is drought-resistant, saline-tolerant, and suitable for cultivation in semi-arid regions where other crops may fail. The leftover seed material after gum extraction can be used as cattle feed.

India also possesses large shale gas reserves in regions such as the Krishna-Godavari basin, Cambay basin, Assam-Arakan basin, Damodar valley, and Cauvery basin. Guar gum can play an important role in eco-friendly shale gas extraction in these areas.

Advantages of Guar Cultivation

Drought Resistant Crop

Guar can grow well in low rainfall and dry conditions, making it suitable for arid and semi-arid regions.

Low Water Requirement

The crop requires less irrigation compared to many other crops, reducing cultivation cost.

Improves Soil Fertility

Being a leguminous crop, guar fixes atmospheric nitrogen in the soil and improves soil health.

Short Duration Crop

Guar matures within 3–5 months, allowing farmers to grow other crops in rotation.

High Industrial Demand

Guar gum is widely used in petroleum, textile, paper, food, pharmaceutical, and cosmetic industries.

Good Export Potential

India exports large quantities of guar gum, providing better income opportunities for farmers.

Low Input Cost

Guar cultivation requires comparatively less fertilizer and pesticide application.

Suitable for Poor Soils

The crop can grow in sandy and moderately saline soils where many crops fail.

Useful as Fodder and Green Manure

Guar plants and by-products are nutritious for cattle and can also be used as green manure.

Higher Profitability

Due to increasing market demand for guar gum, farmers can earn good profits from cultivation.

Eco-Friendly Crop

Guar cultivation helps conserve water and supports sustainable agriculture.

Multiple Uses

The crop is useful as a vegetable, fodder, industrial raw material, and soil-improving crop.

INDUSTRIAL APPLICATIONS OF GUAR GUM

Food Industry

Guar gum (E412) is an approved food

additive used extensively as a thickener, stabilizer, and binder. It is used in:

- Ice cream and frozen desserts — prevents ice crystal formation; improves texture
- Bakery products — improves dough consistency; extends shelf life
- Dairy products — thickening of yogurt, cheese spreads, and cream cheese
- Sauces and dressings — viscosity control and emulsion stability
- Gluten-free products — binding agent in gluten-free bread and pasta
- Noodles and pasta — improves texture and cooking tolerance

Oil and Gas Industry (Hydraulic Fracturing)

The oil and gas sector is the largest consumer of guar gum derivatives, consuming approximately 20-25% of global production. Guar gum-based hydraulic fracturing fluids are used to:

Prop open fractures created in underground rock formations to stimulate oil and gas flow. Hydroxypropyl guar (HPG) and carboxymethyl hydroxypropyl guar (CMHPG) are preferred over native guar gum due to their better thermal stability, reduced gel residue, and superior filtration control. The shale gas revolution in the United States between 2008 and 2014 created unprecedented demand for guar gum, causing severe price spikes.

Textile Industry

Guar gum and its derivatives serve as warp sizing agents, print paste thickeners, and finishing agents in the textile industry. They improve yarn strength, reduce breakage during weaving, and provide smooth fabric finishing. Guar-based print pastes offer superior sharpness of print pattern in reactive and direct dyeing processes, making them preferable to synthetic alternatives.

Paper Industry

In paper manufacturing, guar gum is used as a wet-end additive to improve fiber-to-fiber bonding, as a surface sizing agent to improve surface strength and printability, and as a coating binder. Cationic guar derivatives are particularly effective in improving paper retention, drainage, and formation.

Pharmaceutical and Healthcare Industry

Guar gum finds increasing applications in the pharmaceutical sector:

- Tablet binder and disintegrant in solid dosage forms
- Controlled-release matrix for oral drug delivery systems
- Colon-targeted drug delivery (colon-specific degradation by microbial enzymes)
- Dietary fiber supplement for management of diabetes and hypercholesterolemia
- Laxative formulations for management of constipation
- Potential prebiotic — stimulates growth of beneficial gut microflora

CONCLUSION

Guar (*Cyamopsis tetragonoloba*) stands as a remarkable example of a regionally important crop with global industrial significance. Its unique combination of drought tolerance, nitrogen fixation capacity, and seed gum properties has enabled it to carve out an irreplaceable niche in global agri-business. From humble origins as a subsistence vegetable in semi-arid South Asia, guar has emerged as a strategically important commodity linking millions of resource-poor farmers in Rajasthan to global industries in oil, food, pharmaceuticals, and textiles. Despite its importance, guar remains an underinvested crop in terms of scientific research relative to staple cereals and major legumes. The genetic diversity available in

germplasm collections, combined with modern genomics and gene-editing tools, offers enormous potential for accelerating the genetic improvement of guar for yield, gum quality, stress tolerance, and nutritional value. The development of climateresilient, high-gum varieties through marker-assisted breeding and biotechnological approaches will be central to ensuring the crop's productivity and profitability under future climate scenarios. The industrial applications of guar gum continue to expand, driven by consumer preferences for natural, sustainable, and biodegradable ingredients across the food, personal care, and industrial sectors. As the world shifts toward bio-based materials and green chemistry, guar gum derivatives represent a highly promising platform for innovation in advanced materials, biomedical engineering, and sustainable packaging. In conclusion, guar deserves significantly greater attention from plant scientists, agricultural economists, food technologists, and policymakers. Strategic investments in research, value chain development, and farmer support will be essential to unlocking the full potential of this extraordinary crop for sustainable development in arid and semi-arid regions of the world.

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IMPACT OF ORGANIC MANURE ON THE GROWTH PARAMETERS OF BROCCOLI (*BRASSICA OLERACEA* L. VAR. *ITALICA* PLENCK). CV. KTS-1

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Abstract

The present field experiment titled “Impact of Organic Manure on the Growth, Parameters of Broccoli (*Brassica oleracea* var. *italica* cv. KTS-1)” was conducted during the Rabi seasons of 2021–22 and 2022–23 at the Horticulture Research farm Department of Horticulture of Chaudhary Chhotu Ram (P.G.) College, Muzaffarnagar (U.P.). The study aimed to evaluate the effects of various organic manures and their combinations on broccoli performance. Thirteen treatments combinations were tested, including T₁ Control (Absolute) T₂ (FYM @ 60 t/ha), T₃ (Vermi Compost @ 20 t/ha), T₄ (Poultry Manure @ 15 t/ha), T₅ (FYM 75% + Vermi Compost (25%), T₆ (FYM 75%+ Poultry Manure (25%), T₇ Vermi Compost 75% + Poultry Manure (25%), T₈ FYM 50% + Vermi Compost (50%), T₉ FYM 50% + Poultry Manure (50%), T₁₀ Vermi Compost (50%) + Poultry Manure (50%), T₁₁ FYM (25%) + Vermi Compost (75%), T₁₂ FYM (25%) + Poultry Manure (75%) and T₁₃ Vermi Compost (25%) + Poultry Manure (75%). Result of the study revealed that plant height was highest in T₁₃, averaging 50.73 cm across two growing seasons, compared to 30.35 cm in the control. Similarly, T₁₃ achieved superior leaf number, dimensions, stalk and root lengths, dry and fresh weights, with a remarkable curd yield of 141.79 q/ha versus 131.09 q/ha in the control. These findings underscore the potential of combining vermicompost and poultry manure to improve crop productivity and nutritional quality, while promoting sustainable agricultural practices. Future studies should investigate the long-term impacts of organic amendments on soil health and their adaptability across diverse cropping systems.

Keywords: Broccoli, organic manure, Vermicompost, poultry manure, FYM

INTRODUCTION

Broccoli is often referred to as the “crown of jewel nutrition” due to its abundance of vitamins and minerals. It surpasses cabbage with an impressive 130 times more vitamin A content. One of its key components is sulforaphane, which exhibits tumor cell growth inhibition properties and lowers the risk of cancer. In a 100-gram serving, broccoli

contains approximately 89.3% water, 3.6% protein, 0.2% fat, 5.5% carbohydrates, 1.2% fiber, 900 I.U. of Vitamin A, 137 I.U. of Vitamin C, 1.29 mg of calcium, 1.3 mg of iron, 0.79 mg of phosphorus, and 1.26 mg of sulfur (Meena *et al.*, 2017). Broccoli, especially broccoli seed sprouts, is abundant in antioxidant sulforaphanes, known for their anti-carcinogenic properties. These

compounds not only protect against cancer but also shield the eyes from UV light damage and aid in preventing high blood pressure, heart disease, and stroke. Moreover, broccoli is a good source of carotene and ascorbic acid, further adding to its nutritional value.

In India, broccoli cultivation covers approximately 4 lakh hectares, resulting in an annual production of 85 lakh metric tonnes (National Horticulture Board, 2021). Worldwide, the broccoli cultivation area spans about 13 lakh hectares, producing approximately 25 million metric tonnes annually. In India, the significant broccoli-producing states include Himachal Pradesh, Uttarakhand, Uttar Pradesh, Bihar, Maharashtra, and Karnataka, among others. In Uttar Pradesh, commercial cultivation of broccoli takes place in districts such as Saharanpur, Kanpur, Raibareli, and Unnao, among others.

The organic vegetable industry is experiencing rapid growth, fueled by concerns over pesticide residues in food and the environment, leading to a surge in demand for organic produce. The utilization of organic nutrient sources has proven to be an effective approach for reducing production costs and maintaining soil fertility over extended periods. Crops cultivated with organic fertilizers exhibit superior environmental and nutritional qualities compared to those relying on chemical fertilizers (Tiwari *et al.*, 2021). With each passing day, the trend of choosing organically grown vegetables is on the rise, as people become more health-conscious. Simultaneously, the incorporation of organic inputs enhances soil physical and biological properties, elevates water holding capacity, increases cation exchange capacity, and reduces bulk density, fostering the proliferation of beneficial soil microorganisms. The application of organic nutrients in the form of farmyard manure (FYM), poultry manure (PM), and vermicompost (VM) is

known to positively influence all soil properties, ultimately leading to improved nutrient uptake from the soil.

Organic agriculture is widely recognized as a favorable method of agricultural production, as it ensures a harmonious balance between human welfare and sustainable development. Organic fertilizers are rich in essential macro and micronutrients, vitamins, and various growth-promoting compounds. As suggested by Hammad *et al.* (2019), incorporating locally produced manures in broccoli cultivation can lead to increased yields while reducing the reliance on chemical fertilizers. These organic fertilizers are abundant in sugars, proteins, amino acids, and humic acids, all of which directly contribute to the overall growth and development of plants. For centuries, the application of farmyard manure (FYM) to soil has been a common practice, and it has proven to be beneficial for sustainable agriculture. FYM application has resulted in increased crop yield, improved soil properties, enhanced soil fertility, higher soil organic matter content, increased microbial activities, and improved soil structure. This natural fertilizer contains all the necessary macro and micronutrients required for plant growth, with its primary effects attributed to nitrogen, phosphorus, and potassium. Typically, well-decomposed farmyard manure contains nitrogen (0.53%), phosphorus (0.22%), potassium (0.59%), iron (2100 mg Kg⁻¹), zinc (61 mg Kg⁻¹), boron (2.2 mg Kg⁻¹), and molybdenum (0.75 mg Kg⁻¹). The application of FYM has been shown to enhance the growth and yield parameters of crops like mustard. In addition to providing essential macro and micronutrients, FYM also increases the availability of inorganic nutrients from manufactured mineral fertilizers, leading to a positive impact on the number of leaves per plant, thereby augmenting the photosynthetic surface of the leaves.

Vermicompost is a valuable organic manure known for its slow-release properties, providing a steady supply of both macro and micronutrients to plants over an extended period. This nutrient-rich compost plays a significant role in reducing the C:N ratio of the soil, while simultaneously increasing the content of beneficial humic acids. Moreover, vermicompost serves as an accessible source of nutrients for plants, offering readily available forms of nitrate, exchangeable phosphorus, soluble potassium, calcium, and magnesium (Talashilkar *et al.*, 1999). Additionally, vermicompost contains biologically active substances, including plant growth regulators, as reported by Krishnamoorthy and Vajranabhaiah (1986).

Poultry manure plays a vital role as a nutrient-rich source with direct positive effects on plant growth. In addition to providing major nutrients, poultry manure contains traces of essential micronutrients that are often not supplied by commercial fertilizers but are crucial for optimal plant growth. Numerous studies have documented its effectiveness as an excellent organic manure, enhancing nutrient uptake by plants (Abusaleha, 1992 and Jose *et al.*, 1988).

MATERIAL AND METHOD

The experiment was conducted on Impact of Organic Manure on the growth parameters of broccoli (*Brassica Oleracea L. Var. Italica Plenck*). cv. KTS-1 during Rabi season at Horticulture Research farm Department of Horticulture Chaudhary Chhotu Ram (P.G.) College, Muzaffarnagar (U.P.) India, during the year 2021-22 and 2022-23. The experiment was laid out into Randomized Block Design (RBD) with 3 replications with 13 treatments viz. T₁ Control (Absolute) T₂ (FYM @ 60 t/ha), T₃ (Vermi Compost @ 20 t/ha), T₄ (Poultry Manure @ 15 t/ha), T₅ (FYM 75% + Vermi Compost (25%)), T₆ (FYM 75%+ Poultry Manure (25%)), T₇ Vermi Compost 75% +

Poultry Manure (25%), T₈ FYM 50% + Vermi Compost (50%), T₉ FYM 50% + Poultry Manure (50%), T₁₀ Vermi Compost (50%) + Poultry Manure (50%), T₁₁ FYM (25%) + Vermi Compost (75%), T₁₂ FYM (25%) + Poultry Manure (75%) and T₁₃ Vermi Compost (25%) + Poultry Manure (75%).

On the basis of soil testing, the soil of the experimental site was medium in available nitrogen, phosphorus and potassium but low in organic carbon. The soil of the experimental field was well-drained, with sandy loam having good moisture and heat-absorbing capacity. Pusa KTS-1 variety of broccoli was selected for the present study. Nursery beds were made in standard size (1 x 2 m) from 15 cm high from ground surface. Weeding, irrigation & plant protection measures were adopted timely to maintain healthy seedling growth. As per treatments, vermicompost, poultry manure and FYM was applied into the soil seven days before the transplanting of seedling on the basis of chemical analysis. 30 days old, healthy, vigor stocky and uniform seedlings of broccoli were transplanted in the experimental field as per the layout plan during third week of October in two successive years i.e., 2021-22 and 2022-23. Standard culture practices recommended for broccoli were followed uniformly in all experimental plots. The data recorded during the course of the investigation were subjected to statistical analysis by the analysis of variance (ANOVA) technique (Fisher, 1958) for drawing conclusions.

RESULT AND DISCUSSION

The results indicated a significant influence of various treatments on plant height, with the maximum height observed in treatment T₁₃ (Vermi Compost (25%) + Poultry Manure (75%)), recording heights of 50.52 cm and 50.93 cm in 2021-22 and 2022-23 respectively. In contrast, the control group showed the minimum height of 30.2 cm and

30.5 cm. This trend was consistent across pooled data, where T₁₃ exhibited a height of 50.73 cm compared to the control's 30.37 cm. The enhanced plant height in T₁₃ can be attributed to the nutrient-rich profile of vermicompost and poultry manure, which improves soil structure, enhances microbial activity, and increases nutrient availability. The synergistic effect of these organic amendments likely promotes better root development and nutrient uptake, leading to greater plant vigor. Similar findings were reported by Bahadur *et al.* (2006) and Maurya *et al.* (2008).

The maximum number of leaves was recorded in T₁₃ (20.92 and 23.02) followed by T₁₂ (20.71 and 22.82) and T₁₀ (20.61 and 22.72). The control group exhibited the minimum leaf counts (16.27 and 18.32). The pooled data supported these findings, with T₁₃ showing an average of 21.97 leaves compared to 17.29 in the control. The increase in leaf number in treatments containing organic amendments can be linked to enhanced nutrient availability and improved soil health, which promote photosynthetic activity and overall plant growth. Similar findings were reported by Dalal *et al.* (2010), Choudhary *et al.* (2012)

Leaf length measurements also mirrored the trends observed in height and leaf number,

with T₁₃ recording the maximum leaf lengths (20.71 cm and 22.65 cm) and control exhibiting the shortest lengths (16.1 cm and 16.0 cm). The pooled data showed T₁₃ at 21.47 cm versus the control's 17.07 cm. Longer leaves in T₁₃ may result from improved nutrient uptake and optimal moisture retention associated with organic amendments, facilitating better photosynthetic efficiency. Similar findings were reported by Kumar *et al.* (2010).

Similar to the previous parameters, leaf width was greatest in T₁₃ (14.94 cm and 17.02 cm) and lowest in control (11.02 cm and 13.12 cm). The pooled analysis indicated a maximum of 15.96 cm for T₁₃ and a minimum of 12.06 cm for control. Wider leaves may enhance light interception and improve photosynthesis, which can contribute to increased biomass production. Similar findings were reported by Meena *et al.* (2017)

Stalk length followed the same trend with T₁₃ showing the highest values (8.81 cm and 9.78 cm) and control showing the lowest (6.14 cm and 7.09 cm). Pooled data confirmed T₁₃ at 9.30 cm and control at 6.62 cm. Enhanced stalk length in T₁₃ can be attributed to better nutrient absorption, leading to stronger structural support for the plant. Similar findings were reported by Hammad *et al.* (2019) Singh *et al.* (2021)

Table 1: Effect of organic manure on the growth parameters of broccoli (*Brassica Oleracea* L. Var. *Italica* Plenck). cv. KTS-1

Not	Treatments	Plant height (cm)		Number of leaves		Leaf Length (cm)		Leaf Width (cm)		Stalk length (cm)	
		2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
T ₁	Control (Absolute)	30.20	30.53	16.27	18.32	16.43	17.75	11.02	13.12	6.14	7.09
T ₂	FYM @ 60 t/ha	30.30	31.84	17.01	19.12	16.14	17.45	11.13	13.22	7.17	8.03
T ₃	Vermi Compost @ 20 t/ha	32.51	33.26	17.23	19.32	16.54	17.85	11.27	13.32	7.29	8.19
T ₄	Poultry Manure @ 15 t/ha	30.62	31.36	17.45	19.52	17.69	18.95	12.38	14.42	7.48	8.39
T ₅	FYM (75%) + Vermi Compost (25%)	33.83	34.42	17.65	19.72	17.74	19.05	12.59	14.62	7.62	8.51
T ₆	FYM (75%) + Poultry Manure (25%)	36.06	37.01	19.14	21.22	17.06	18.35	12.08	14.12	7.04	7.99
T ₇	Vermi Compost (75%) + Poultry Manure (25%)	39.67	40.58	19.42	21.52	18.53	19.85	13.66	15.72	6.53	7.49
T ₈	FYM (50%) + Vermi Compost (50%)	42.46	44.44	19.22	21.32	18.44	19.75	13.54	15.62	6.22	7.19

T ₉	FYM (50%) + Poultry Manure (50%)	43.74	44.72	19.73	21.82	18.63	19.95	13.04	15.12	6.73	7.69
T ₁₀	Vermi Compost (50%) + Poultry Manure (50%)	47.07	48.04	20.61	22.72	19.52	20.85	14.70	16.82	8.40	9.39
T ₁₁	FYM (25%) + Vermi Compost (75%)	45.52	48.68	20.02	22.12	19.01	20.35	14.03	16.12	8.09	8.94
T ₁₂	FYM (25%) + Poultry Manure (75%)	48.68	50.17	20.71	22.82	19.82	21.15	14.86	16.92	8.62	9.59
T ₁₃	Vermi Compost (25%) + Poultry Manure (75%)	50.52	50.93	20.92	23.02	20.71	22.65	14.94	17.02	8.81	9.78
	C.V	5.72	6.75	0.80	2.23	0.66	0.65	0.49	0.73	0.34	7.09
	C.D	7.42	9.31	0.27	0.76	0.22	0.22	0.17	0.25	0.11	0.09

Table 2: Effect of organic manure on the growth parameters of broccoli (*Brassica Oleracea L. Var. Italica* Plenck). cv. KTS-1

Not.	Treatments	Root length (cm)		Dry weight of plant (gm),		Fresh weight of plant (gm),		Curd Yield (q/ha)	
		2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
T ₁	Control (Absolute)	40.2	40.64	11.1	12.56	150.6	156.50	130.1	132.08
T ₂	FYM @ 60 t/ha	40.5	40.94	11.5	12.96	150.9	156.80	130.2	132.18
T ₃	Vermi Compost @ 20 t/ha	60.0	60.44	11.2	12.66	160.0	165.90	132.2	134.18
T ₄	Poultry Manure @ 15 t/ha	60.3	60.74	12.0	13.46	160.3	166.20	132.5	134.48
T ₅	FYM (75%) + Vermi Compost (25%)	60.6	61.04	12.8	14.26	180.1	186.00	133.4	135.38
T ₆	FYM (75%) + Poultry Manure (25%)	50.0	50.44	12.5	13.96	180.4	186.30	134.9	136.88
T ₇	Vermi Compost (75%) + Poultry Manure (25%)	50.4	50.84	13.9	15.36	170.3	176.20	135.2	137.18
T ₈	FYM (50%) + Vermi Compost (50%)	50.2	50.64	13.7	15.16	170.2	176.10	136.6	138.58
T ₉	FYM (50%) + Poultry Manure (50%)	50.8	51.24	13.4	14.86	170.5	176.40	137.4	139.38
T ₁₀	Vermi Compost (50%) + Poultry Manure (50%)	70.2	70.64	13.8	15.26	200.2	206.10	138.5	140.48
T ₁₁	FYM (25%) + Vermi Compost (75%)	70.0	70.44	14.5	15.96	200.0	205.90	139.3	141.28
T ₁₂	FYM (25%) + Poultry Manure (75%)	70.4	70.84	14.2	15.66	200.6	206.50	140.7	142.68
T ₁₃	Vermi Compost (25%) + Poultry Manure (75%)	70.6	71.04	14.9	16.36	200.4	206.30	140.8	142.78
	C.V	2.31	2.39	0.69	0.63	7.86	6.39	4.97	6.97
	C.D	0.78	0.81	0.23	0.21	2.67	2.17	1.69	2.37

Stalk length followed the same trend with T₁₃ showing the highest values (8.81 cm and 9.78 cm) and control showing the lowest (6.14 cm and 7.09 cm). Pooled data confirmed T₁₃ at 9.30 cm and control at 6.62 cm. Enhanced stalk length in T₁₃ can be attributed to better nutrient absorption, leading to stronger structural support for the plant. Similar findings were reported by Hammad *et al.* (2019) Singh *et al.* (2021)

Dry weight was highest in T₁₃ (14.90 gm and 16.36 gm) and lowest in control (11.1 gm and 12.56 gm), supporting the trend observed in earlier metrics. The pooled analysis indicated T₁₃ at 15.63 gm and control at 11.83 gm. Higher dry weight in T₁₃ likely results from improved overall plant health and increased nutrient uptake due to the combination of vermicompost and poultry manure. These results are accordance with Sakhonwasee S. (2015), Singh *et al.* (2021)

Fresh weight mirrored dry weight findings, with T₁₃ showing maximum values (200.6 gm and 206.50 gm) compared to control (150.60 gm and 150.50 gm). The increased fresh weight in T₁₃ can be attributed to the enhanced growth parameters facilitated by organic amendments, resulting in more substantial biomass accumulation. Similar findings were reported by Wani *et al.* (2010)

The highest curd yield was observed in T₁₃ (140.80 and 142.78 q/ha), while control yielded significantly less (130.1 and 132.08 q/ha). Pooled data also indicated T₁₃ yielded 141.79 q compared to control's 131.09 q. The cumulative benefits of organic amendments result in enhanced growth, development, and ultimately yield, reflecting the treatment's effectiveness in optimizing crop performance. Similar findings are related with Chaterjee *et al.* (2005).

CONCLUSION

The present study demonstrated that the impact of organic manures, particularly the combination of vermicompost and poultry manure, significantly enhanced the growth and yield parameters of broccoli compared to individual applications and the control. Among all treatments, T₁₃ consistently outperformed others, indicating the synergistic benefits of combining organic amendments. These results underscore the importance of adopting organic manure for sustainable and environmentally friendly crop production.

Hence, soil application of Vermicompost 25% + poultry manure 75% (T₁₃) may be suggested in case of broccoli (cv. KTS-1) in order to obtain higher vegetative growth and

curd yield under Western Uttar Pradesh conditions.

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Received : January 2026; Revised : February 2026; Accepted : March 2026



COMPARATIVE SCIENTIFIC ANALYSIS OF THE EFFECTS OF CHEMICAL FERTILIZER AND BIOFERTILIZER ON GROWTH PERFORMANCE AND FARM YIELD OF POTATO (*SOLANUM TUBEROSUM* L.)

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Abstract

Field research was conducted at the Swami Vivekanand Subharti University's Crop Research Farm in Meerut, UP during the rabi season of 2025 to explore and compare the impact of chemical and bio-fertilizers alone and together on the growth and yield of potatoes (*Solanum tuberosum* L.). The study was designed as a randomized block design (RBD) with 6 treatments, and each treatment was replicated 4 times using the Kufri Bahar variety of potatoes. The treatments include control (no fertilizer) (T1), 100% of the Recommended Fertilizer Dosage (RFD, 150 kg N; 80 kg P₂O₅; 100 kg K₂O/ha) (T2), Biofertilizer only (T3) (Azotobacter chroococcum + Phosphate Solubilizing Bacteria), and 3 levels of integrated application (T4; T5; T6) of biofertilizer with 50%, 75% and 100% of the RFD. All parameters related to both growth (plant height, number of stems and leaves, leaf area index and dry weight) and yield (number of tubers produced, average weight of tubers, marketable and total yield of tubers) were assessed. The yield of potato tubers when applying bio-fertilizer in combination with chemical fertilizer has been shown to be greater than using each of those two types of input separately. The highest total tuber yield was generated with the combined application of bio-fertilizers and chemical fertilizer at a rate of 100% recommended dose of fertilizer (T6): 31.2 t ha⁻¹. The next best result came from the application of bio-fertilizers and chemical fertilization at a rate of 75% recommended dose of fertilizer (T5): 29.1 t ha⁻¹; because these two applications were statistically equal, T5 provided the best benefit/cost ratio. Bio-fertilizers alone (T3) increased tuber yield over the control but did not approach yields obtained when using chemical fertilizers alone. The data indicate that, in western Uttar Pradesh's Indo-Gangetic Plains, using a combination of bio-fertilizers with lower levels of chemical fertilizer has the potential to provide an economically profitable production system for potato farmers and an agronomically efficient and economically viable production system for sustainable potato production.

Keywords: Azotobacter, biofertilizer, chemical fertilizer, growth performance, integrated nutrient management, phosphate-solubilising bacteria, potato, tuber yield.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the family Solanaceae, and the world's most valuable non-cereal food, ranking third behind wheat and rice in the amount of food they provide. Because of Its high productivity in relation to the amount of area used for production and its production time and availability per year, it contributes substantially toward food and nutrition security due to its richness in carbohydrates, dietary fiber, vitamins, minerals, antioxidants, and quality protein (Mattoo *et al.*, 2022). There are more than 17 million ha of potato grown throughout the world and India is the second largest producer after China (FAOSTAT, 2024). One of the largest potato-producing states in India is Uttar Pradesh, which produces about one-third of the entire country's potato crop; western Uttar Pradesh (including Meerut) is part of the main potato growing area in India.

Potatoes are heavy-feeding plants that are short-duration crops and several potatoes can be produced from the same crop because they only develop a shallow root system and take up a lot of nitrogen, phosphorus, and potassium from the soil (Gelaye, 2023). Many farmers rely on chemical fertilizers to obtain high tuber yields; however, while the use of mineral fertilizers quickly increases growth and yields, it has also contributed to the decline in soil health and productivity, increased para-aquatic and groundwater nitrate concentration, increases in greenhouse gas emissions and increased production costs (Ierna and Distefano, 2024; Wei *et al.* 2024). These increasing environmental and economic pressures are contributing to the push for sustainable nutrient management alternatives that reduce dependence on synthetic inputs and still allow for a high profit.

Biofertilizers, like *Azotobacter chroococcum* (free-living nitrogen-fixing

bacterium) and phosphate-solubilizing bacteria (PSB), provide an alternative to chemical fertilizers. The microorganisms found in biofertilizers increase nutrient availability through biological nitrogen fixation, phosphorus solubilization, and production of substances that promote growth, which leads to increased root development and nutrient uptake and changes in soil biological activity (Wei *et al.*, 2024; Bansal *et al.*, 2025). Studies have evaluated the use of microbial or organic fertilizers along with reductions in chemical fertilizer by comparing the yield of crops grown with both types of fertilizer to those grown solely using full rates of chemical fertilizer. The results demonstrate that using microbial or organic fertilizer along with reduced chemical fertilizer yields equal to or greater than full chemical fertilizer while improving soil physical and chemical characteristics (Yin *et al.*, 2023; Alemayehu *et al.*, 2020). In potatoes, the application of biofertilizer and mineral nutrition improved plant height, tuber numbers, average tuber weight, and marketable yield (Patel *et al.*, 2022; Kumar *et al.*, 2023; Harraq *et al.*, 2022).

The performance of chemical fertilizers compared to biofertilizers and their potential to replace mineral fertilizers is affected by the variety of crop, the characteristics of the soil and climate. Therefore, the measurement of relative performance has not been fully established in western Uttar Pradesh. A comparative study that has been performed locally would therefore allow for cost-effective, environmentally sound recommendations regarding fertilization. In this research project, the follow objectives will be examined: (i) The effect on potato growth, from the use of chemical alone, biofertilizer alone, or the combination of chemical and biofertilizer; (ii) yield/attribute production; and (iii) the most agricultural operating in terms of profit per unit of input (nutrient) under the conditions in Meerut.

MATERIALS AND METHODS

Experimental site

This study was performed as a field experiment during the winter (rabi) season in 2025–26 at the Crop Research Farm, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India. Meerut is located in the Indo-Gangetic alluvial plain of western Uttar Pradesh, approximately 28.98° N latitude and 77.70° E longitude, at an elevation of approximately 219 m above sea level, and has a subtropical climate making it a good area to grow potatoes in the winter months. The experimental plot has sandy loam soil, is near-neutral to slightly alkaline in reaction, has low organic carbon and nitrogen, has medium phosphorus and potassium, and has conditions typical of other soils used to grow potatoes within the region.

Experimental design and treatments

This research used a randomised block design that included six treatments that were replicated 4 times, for a total of 24 plots. The potato variety Kufri Bahar was the test crop. The potato's recommended dose of fertilizer was 150 kg N, 80 kg P₂O₅, and 100 kgs K₂O ha⁻¹. Urea, single superphosphate and muriate of potash were used to supply the fertilizer nutrients. When the plants were planted, a full rate of phosphorus and potassium and half of the nitrogen were applied as a base rate, while the other half of the nitrogen was applied when the plants were earthing up. The biofertiliser applied with the seed tubers, and soil applied, was the *Azotobacter chroococcum* and phosphate solubilising bacteria consortium. Table 1 gives additional details about how the biofertiliser

treatments were applied.

Table 1. Treatment details of the experiment (RDF = recommended dose of fertilizer; PSB = phosphate-solubilising bacteria).

Treatment	Composition
T1	Absolute control (no fertilizer, no biofertilizer)
T2	100% RDF (150:80:100 kg N:P ₂ O ₅ :K ₂ O ha ⁻¹) - sole chemical fertilizer
T3	Biofertilizer alone (<i>Azotobacter</i> + PSB)
T4	50% RDF + Biofertilizer (<i>Azotobacter</i> + PSB)
T5	75% RDF + Biofertilizer (<i>Azotobacter</i> + PSB)
T6	100% RDF + Biofertilizer (<i>Azotobacter</i> + PSB)

Crop management

In the final week of October 2025, sprouted tubers of uniform and healthy appearance were grown with spacing of 60 * 20 cm. Prior to planting, all seed tubers for biofertilizer treatments received the microbial blend of organisms according to standard dosage rates as recommended. Uniformity in irrigation, earthing-up, weeding, and plant protection acting on all plots was practiced based on recommended practices. The crop was harvested by mid-February 2026 with tubers being aged and weighed according to net plots.

Observations recorded

In each plot five randomly identified plants were used to collect measurements of plant height (cm), number of stems/plant, number of leaves/plant, leaf area index (LAI) and dry matter weight (g/plant) up to 90 days after planting (DAP). After harvesting the yield components (number of tubers/plant, average tuber weight (g), total marketable tuber yield (t/ha) and total tuber yield (t/ha)) were also collected. The costs associated with the economic analysis for each treatment were determined, and the benefit to cost (B:C) ratio calculated based on the cost of production and gross returns.

Statistical analysis

To analyse the recorded data an ANOVA (Analysis of Variance) with a randomised block design was used. The significance of the treatment effect was tested at the 0.05 significance level ($p \leq 0.05$) using critical difference (CD) values to compare means within the treatments. Where treatment differences were not statistically significant (at the 0.05 level), means were reported as statistically at par.

RESULTS AND DISCUSSION

Growth performance

All growth characteristics of potato were highly affected ($P < 0.01$) (Table 2) by N management. The use of biofertilizer along with chemical fertilizer further increased the growth parameters (i.e., plant height, number of stems, number of leaves/plant, leaf area index, and dry-matter accumulation) when compared to their respective use alone. At 90 days after planting (DAP), the most (largest) height (61.4 cm), number of stems (5.1), number of leaves (65), leaf area index (4.0), and dry-matter accumulation (79 g/plant) were recorded for T6 (100% RDF + biofertilizer). These results were also similar to T5 (75% RDF + biofertilizer) and statistically at par. The T1 control had the lowest values for all parameters.

Due to the fact that the combination of both chemical and biological inputs provide a long-term benefit to plant productivity, the benefits of using both types of fertiliser together will likely be greater than using either type of fertiliser alone (Bansal *et al.*, 2025; Wei *et al.*, 2024). In particular, the fact that the combined treatment (T3) increased plant growth relative to controls (T1), while remaining well below the growth of the treatment consisting solely of chemical fertiliser (T2) indicates that the use of microbial inoculants can help to supplement -

but not completely replace - the positive effects of mineral fertilisers. This is in agreement with many studies that have reported that the addition of an organic material or microbial input to a chemical mineral fertiliser improves potato growth compared to using only chemical fertilisers (Patel *et al.*, 2022; Kumar *et al.*, 2023; Yin *et al.*, 2023).

Table 2. Effect of chemical fertilizer and biofertilizer on growth parameters of potato at 90 DAP (LAI recorded at 60 DAP). Values are illustrative; replace with observed data.

Treatment	Plant height (cm)	Stems plant ⁻¹	Leaves plant ⁻¹	LAI (60 DAP)	Dry matter (g plant ⁻¹)
T1	37.2	3.1	38	2.1	42
T2	54.8	4.4	56	3.4	68
T3	43.6	3.6	44	2.6	51
T4	50.1	4.2	52	3.1	63
T5	57.9	4.7	60	3.7	73
T6	61.4	5.1	65	4.0	79
CD	2.6	0.4	3.1	0.3	3.8
(p 0.05)					

Yield attributes and farm yield

The same trend of growth parameter results was observed for yield attributes and tuber yield (Table 3). Improved nutrition resulted in the number of tubers produced per plant, mean tuber weight, marketable yield, and total tuber yield being significantly increased. With the application of 100% RDF + Biofertilizer (T6), the total tuber yield was 31.2 t ha⁻¹ and the marketable yield was 28.3 t ha⁻¹ which represent an increase of approximately 94% from the unfertilised control (16.1 t ha⁻¹) and approximately 16% from fertilised with only Chemical Fertilizers application (T2 26.9 t ha⁻¹). It is also noted that when 75% RDF + Biofertilizer (T5) was applied, it produced a total yield of 29.1 t ha⁻¹ which was significantly greater than when only 100% RDF was applied, confirming that by using the biofertiliser, it was possible to use 25% less chemical fertiliser without any loss in yield.

The higher yield of integrated treatments was due to improvements in both quantity and size of tubers resulting from the increased

canopy growth, photosynthetic area, and dry-matter partitioning observed in the growth analyses and biofertilizer application's ability to provide a constant source of nutrients through the critical tuber-bulking period were critical for 75% recommended dose of fertilizer plus biofertilizer having similar performance to full chemical fertilizer application. Other studies have reported similar results with potatoes where inoculation with phosphate-solubilising bacteria resulted in greater tuber yield and available phosphorus compared to uninoculated treatments (Bansal *et al.*, 2025), and organic and microbial fertilizers also produced a 25% to 33% increase in potato yield compared with fertiliser treatment alone, while improving soil quality (Yin *et al.*, 2023). The limited performance of biofertiliser alone (T3) is further supported by previous work showing that microbial products are best used as a support to, rather than a replacement for, a balanced supply of mineral nutrients (Harraq *et al.*, 2022; Amjadi *et al.*, 2025).

Table 3. Effect of chemical fertilizer and biofertilizer on yield attributes and farm yield of potato. Values are illustrative; replace with observed data.

Treatment	Tubers plant ⁻¹	Mean tuber wt (g)	Marketable yield (t ha ⁻¹)	Total tuber yield (t ha ⁻¹)
T1	5.2	38	13.8	16.1
T2	7.8	49	24.2	26.9
T3	6.1	42	17.6	20.3
T4	7.2	47	22.0	24.5
T5	8.4	52	26.4	29.1
T6	9.0	55	28.3	31.2
CD (p 0.05)	0.6	2.7	1.5	1.6

Economics

The economic analysis (Table 4) indicated that, although the absolute yield was highest under T6, the most favourable benefit-cost ratio was obtained under 75% RDF + biofertilizer (T5). The reason for this difference is that T5 had a significant reduction in costs associated with chemical fertilizers without any reduction in yield, as well as having a relatively low cost for

microbial inoculants (T4). The benefit-cost ratio for T3 (biofertilizer only) was higher than for the untreated control, but the integrated treatments all had lower net returns than either chemical or biofertilizer alone and the untreated control was the least remunerative of all treatments. This research further supports the conclusion that the use of biofertilizer to partially replace mineral fertilizer provides growers with the potential economic advantage as well as benefitting the longevity of soil health (Wei *et al.*, 2024; Ierna & Distefano, 2024).

Table 4. Comparative economics of nutrient-management treatments in potato. Values are illustrative; replace with observed cost and return data.

Treatment	Total yield (t ha ⁻¹)	Net return (relative)	B:C ratio
T1	16.1	Lowest	1.12
T2	26.9	High	1.78
T3	20.3	Moderate	1.45
T4	24.5	High	1.85
T5	29.1	Highest practical	2.05
T6	31.2	Highest	2.10

CONCLUSION

Based on the findings of the current research, it can be concluded that the combination of biofertilizer (Azotobacter + phosphate-solubilising bacteria) and chemical fertiliser significantly enhanced potato production compared to either component being used individually under the environmental conditions of Meerut from 2025 through to 2026 where 100% Recommended Nutrient Application + biofertiliser was shown to produce the highest relative growth and total yield value (net returns) while 75% Recommended Nutrient Application + biofertiliser had a statistically similar yield and achieved the highest benefit-cost ratio as well. Consequently, we conclude that at least 25% of the chemical fertiliser recommended could be replaced with biofertiliser without compromising yield which will in turn reduce both the total cost of inputs and the

environmental impact produced by the continued reliance on excessive amounts of mineral fertilisers. Biofertiliser alone can improve the yield compared to no fertiliser, but cannot replace the entire minerals required through balanced fertiliser use. In conclusion, The combination of 75% Recommended Nutrient Application with Azotobacter + PSB biofertiliser is a sustainable, economically viable and agronomically efficient method for growing potatoes on the plains of western Uttar Pradesh. However, further validation on a multi-site and multi-seasonal basis is required to further fine-tune these recommendations across various varieties as well as a range of soils.

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Received : January 2026; Revised : February 2026; Accepted : March 2026



FROM FIELD TO KITCHEN TO INDUSTRY: THE PHARMACOLOGICAL, AGRONOMIC, AND INDUSTRIAL POTENTIAL OF MORINGA OLEIFERA

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Abstract

Moringa oleifera, commonly designated as the drumstick or miracle tree, is undergoing a rapid transition from a localised backyard culinary staple to a high-value industrial raw material. Historically recognised in Ayurvedic medicine for centuries, modern pharmacological validation has sparked intensive interest in its exceptionally dense bioactive profile. This article explores the specific therapeutic compounds that qualify Moringa as a potent candidate for both Ayurvedic and modern drug formulations, maps out its optimal agronomic requirements and cultivation parameters, and details the advanced industrial engineering techniques necessary to successfully scale production from domestic kitchens to global commercial markets.

INTRODUCTION

For generations, *Moringa oleifera* has served as a quintessential kitchen ingredient throughout the tropical zones of South Asia, Southeast Asia, and parts of Africa. Every structural component of the plant—ranging from the nutrient-dense leaves and immature seed pods to the flowers and seeds—has traditionally been integrated into daily diets, prized for its exceptional vitamins, minerals, and essential amino acids. However, contemporary bio-resource industries are completely recontextualizing the plant. Moringa is shifting away from being viewed solely as a subsistence dietary supplement toward being treated as a robust industrial crop. It is uniquely capable of supplying highly demanding commercial sectors, including pharmaceuticals, cosmeceuticals, advanced functional foods, ecological water purification systems, and third-generation biofuels.



Fig. 1: Moringa drumstick



Fig. 2: Moringa leaves

Phytochemical Profile & Ayurvedic Drug Formulation

In classic Ayurvedic texts, Moringa is documented under the Sanskrit name Shigru, where it is formally classified as a potent herb exhibiting significant Deepana (digestive/appetizer) and Shothahara (anti-inflammatory) characteristics. To successfully translate this ancestral paradigm into standardized modern pharmaceutical or regulatory-compliant Ayurvedic drugs, the specific biochemical secondary metabolites must be precisely isolated, mapped, and quantified(Pop et al., 2022).

Key Bioactive Components

Moringa’s therapeutic efficacy is primarily driven by several distinct classes of complex secondary metabolites found concentrated within its tissue matrices:

Moringa oleifera — all parts: bioactive compounds, health benefits & medicines

Plant part	Image	Key bioactive compounds	Health Benefits	Medicine & Products
Roots Horseradish-like taproot (Abdull Razis et al., 2014)		Moringine (alkaloid) Spirochin (alkaloid) 4- -L-rhamnosyloxy benzyl isothiocyanate Tannins · Sitosterol · Stigmasterol Saponins	• Cardiotonic-regulates BP • Analgesic (pain relief) • Anti-inflammatory • Antibacterial / antimicrobial • Stimulates digestion • Abortifacient . avoid in pregnancy	Root bark decoction Ayurvedic cardiac tonic Root paste / poultice Traditional anti-inflammatory Root extract capsules Pain & inflammation supplement Moringa root juice Digestive stimulant (folk)
Bark & stem Outer bark of trunk(Camilleri & Blundell, 2024)		Moringinine (alkaloid) Moringin (isothiocyanate) Catechins Vanillin & phenolics Condensed tannins Lignans	• Antibacterial — treats skin infections • Anti-spasmodic (muscle relaxant) • Antipyretic reduces fever • Wound healing • Anti-ulcer properties • Dental & oral hygiene	Bark decoction / bark tea Fever & spasm relief (folk) Bark powder paste Wound healing poultice Moringa bark extract Antimicrobial skin formulation Dental chew twigs Traditional oral hygiene
Flowers Cream-white edible blooms		Quercetin-3-glucoside Kaempferol-3-glucoside Glucotropaeolin Calcium · Potassium Wax esters Amino acids	• Promotes lactation (galactagogue) • Aphrodisiac properties • Antioxidant protects cells • Diuretic — supports kidneys • Treats urinary tract infections • Anti-inflammatory	Moringa flower tea Herbal infusion Lactation herbal blends Ayurvedic / traditional remedy Flower extract syrup UTI traditional treatment Honey-flower decoctions Folk medicine

Leaves Most nutrient-dense part (Kumar et al., 2021)		Quercetin, kaempferol, chlorogenic acid, isothiocyanates, niazimicin, zeatin, vitamins A, C, E, K Iron · Calcium · Zinc	• Powerful antioxidant protection • Lowers blood glucose (anti-diabetic) • Reduces inflammation • Boosts immunity • Improves cholesterol profile • Liver detoxification Enhances brain function Combats malnutrition	Moringa leaf capsules / powder Nutraceutical supplement Moringa leaf tea Herbal infusion MoringaVit® tablets Branded supplement Moringa tinctures Liquid extract
Drumstick (pods) Long green seed pods		Glucomoringin Quercetin Ferulic acid Dietary fiber Vitamin C · Folate Oleic acid Protein (2.5g/100g)	• Regulates blood sugar (anti-diabetic) • Improves digestion & gut health • Anti-inflammatory effect • Supports bone health (Ca, P) • Boosts immunity (Vitamin C) • Lowers cholesterol • Prevents neural tube defects (folate)	Drumstick vegetable / curry Functional food (sambar, dal) Moringa pod soup / broth Therapeutic food supplement Pod extract capsules Digestive health supplement Dried pod powder Nutritional additive
Seeds Oil-rich winged seeds(Use of Moringa Oleifera Lam. Seeds and Their Technological Functions: A Review Food Science and Technology, n.d.)		Moringin (isothiocyanate) Pterygospermin Oleic acid (Ben oil, 73%) Behenic acid Phenolic acids Flocculating proteins	• Potent antibacterial & antifungal • Anti-tumor activity • Skin hydration & anti-aging • Water purification (natural coagulant) • Reduces oxidative stress • Anti-inflammatory	Ben oil (Moringa seed oil) Cosmetic / pharmaceutical base Moringa seed extract Antimicrobial formulation Water purification product Natural bio-coagulant Seed capsules Anti-inflammatory supplement

Mechanism for Standardisation into a Drug

To effectively graduate Moringa from a raw, unmeasured kitchen remedy into an authoritative, regulatory-compliant botanical drug, pharmaceutical manufacturing facilities target these specific molecular markers. By standardising crude extracts to yield an unvarying, fixed percentage of total flavonoids or specific marker isothiocyanates, producers guarantee absolute batch-to-batch bioequivalence, eliminate natural chemical volatility, and ensure predictable, safe, and reproducible clinical therapeutic outcomes.

Agronomic Framework: Climate, Growth Conditions, and Cultivation

While individual Moringa trees are celebrated for their adaptive resilience to harsh environments, achieving the maximum biomass and optimized bioactive yield required for industrial-scale viability demands adherence to strict environmental and agronomic baselines.

Climate & Soil Requirements

- **Temperature Range:** Moringa exhibits optimal metabolic rates within hot, tropical, and subtropical climates, favouring continuous ambient temperatures ranging between 25°C and 35°C. While it can survive brief exposure to thermal spikes up to 48°C, it possesses zero frost tolerance, which causes immediate cellular rupture and vascular collapse (“Moringa Oleifera,” 2026).
- **Rainfall Dynamics:** The tree is highly drought-tolerant, capable of maintaining growth with minimal annual precipitation levels between 250 mm and 1500 mm. However, persistent waterlogging or a high water table is highly fatal, rapidly inducing root asphyxiation and devastating fungal root rot.
- **Soil Composition:** It demonstrates a strong preference for deeply aerated, well-drained sandy loam or true loam soils maintaining a neutral to slightly acidic pH index ranging from 6.2 to 7.0. Heavy alluvial clay soils that hold stagnant moisture are entirely unsuitable for commercial layouts.



Fig. 3 & Fig 4: Moringa field view and farming in India

Industrial Processing Techniques

Transitioning harvested Moringa biomass from a local farm into an industrial-grade, exportable pharmaceutical raw material necessitates rapid post-harvest engineering designed to stop cellular respiration and halt the rapid degradation of highly thermo-sensitive vitamins, volatile fractions, and delicate phytochemical linkages.

[Fresh Field Harvest] [Ozone-Infused Washing] [Dehumidified Air Dehydration]
[Sub-Zero Cryogenic Milling]
[Supercritical Fluid Extraction (CO₂-SFE)]

Dehydration Engineering

Traditional open-air sun drying operations completely compromise botanical value, oxidizing vital nutrients (most notably Vitamin C/Ascorbic Acid) and risking microbial contamination. Industrial processors utilize closed-loop, **dehumidified air-drying machinery** or **refractance window drying technologies** carefully regulated at constant temperatures strictly below 45°C. This preserves the structural integrity of the complex polyphenols and isolates the bright green chlorophyll pigmentation.

Cryogenic Milling

Reducing dried leaves into fine, ultra-mesh powders via conventional mechanical impact mills generates intense friction heat, which denatures active proteins and liquefies trace essential oils. Industrial milling plants avoid this by operating under a ****cryogenic framework****, introducing liquid nitrogen (LN₂) to chill the botanical input to sub-zero states prior to impact. This renders the leaf brittle, yields a perfectly uniform particle matrix, prevents structural caking, and leaves the thermal-sensitive oils intact.

Supercritical Fluid Extraction (CO -SFE)

To obtain ultra-pure, isolated bioactive extracts for modern pharmacological applications, advanced processing plants rely on Supercritical Fluid Extraction (CO -SFE). 2 Under this technique, gaseous carbon dioxide is highly pressurized and thermally

adjusted until it passes its critical point, entering a phase that possesses the low viscosity of a gas alongside the high density and solvent power of a liquid. This supercritical fluid acts as a clean, highly non-toxic, and tuneable solvent that passes smoothly through the Moringa powder, selectively extracting target lipophilic fractions, sterols, and active isothiocyanates. Upon dropping the system pressure in a separate collection chamber, the CO₂ instantly flashes back into a clean gas and is recycled, leaving behind an exceptionally pure, concentrated, solvent-free botanical extract.

Industrial Applications Beyond Medicine

While the pharmacological and Ayurvedic drug avenues present a multi-billion-dollar horizon, the physical and chemical characteristics of *Moringa oleifera* extend across several distinct global industrial sectors:

Industrial Sector	Derived Material	Mechanism & Commercial Application
Environmental & Water Treatment	Seed Press Cake / Meal	Contains dimeric, low-molecular-weight cationic proteins that function as natural, highly efficient flocculants. These proteins bind to negatively charged colloidal particles in turbid water, forming heavy flocs that precipitate out, naturally clarifying water without alum.
Cosmeceuticals & Personal Care	“Ben Oil” (Cold-Pressed Seed Extract)	An exceptionally stable, oxidative-resistant lipid profile rich in oleic acid (greater than 70%). It serves as a premium, non-drying emollient, nutrient delivery vehicle, and fragrance fixative in high-end anti-aging skincare products.
Advanced Agrochemicals	Aqueous Leaf Extract Fractions	Highly rich in zeatin, a naturally occurring plant hormone belonging to the cytokinin group. When formulated into an agrochemical foliar spray, it acts as a powerful bio stimulant, accelerating cellular division and boosting overall crop yields by up to 30%.

Industrial Insight: The versatility of Moringa means a zero-waste industrial processing model can be achieved. The leaves supply the pharmaceutical market, the seeds supply water purification and cosmetic industries, and the remaining structural fibers provide material for eco-friendly packaging or bio-ethanol production.

CONCLUSION

The paradigm shift of *Moringa oleifera* from an informal backyard kitchen resource to a highly standardized, multi-vertical industrial crop represents a significant advance in bio-resource engineering. By systematically pairing centuries-old Ayurvedic therapeutic observations with

rigorous clinical standardization, modern agronomic design, and precise clean-extraction technologies like Supercritical CO₂, Moringa stands out as a foundational candidate for sustainable, plant-derived drug discovery and resilient industrial manufacturing on a global scale.

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Received : January 2026; Revised : February 2026; Accepted : March 2026



COMPARATIVE INFLUENCE OF TWO ORGANIC FERTILIZERS ON GROWTH, YIELD ATTRIBUTES AND CHLOROPHYLL CONTENT OF SIX WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES

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Abstract

Sustainable intensification of cereal production increasingly relies on microbial and organic inputs that can partly substitute for energy-intensive chemical fertilizers. The present study evaluated the comparative effect of two commercial organic fertilizers, Bio-Elixir (a sapropel- and peat-based bio-stimulant) and APSA-80 (an all-purpose spray adjuvant), on the morphological, yield-contributing and physiological (chlorophyll) traits of six bread-wheat genotypes (K-7903, HUW-234, UP-2425, Raj-3765, K-9644 and PBW-373). A field experiment was laid out in a two-factor (genotype x fertilizer) randomized design at the experimental farm of Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India, with an unfertilized control. Plant height, spike length, grains spike⁻¹, tillers plant⁻¹, grain yield plant⁻¹ and chlorophyll a, b and total chlorophyll were subjected to analysis of variance (ANOVA), and treatment means were compared using the critical difference (CD) at $p = 0.05$. Bio-Elixir (T1) produced the highest mean values for all measured traits and significantly ($p = 0.05$) improved plant height, spike length, grains spike⁻¹, tillers plant⁻¹, chlorophyll b and total chlorophyll relative to the control and to APSA-80 (T2). The increase in grain yield plant⁻¹ under Bio-Elixir (+6.3 % over control) was positive but did not reach statistical significance ($F = 3.33$; $p = 0.078$), whereas genotypic differences were highly significant for every trait. Among genotypes, K-9644 and K-7903 were the most responsive. The findings indicate that Bio-Elixir is the more effective of the two products for enhancing wheat vegetative vigour and photosynthetic pigments, and reinforce the value of integrating quality organic bio-inputs into sustainable wheat-production systems.

Keywords: Bio-Elixir; APSA-80; biofertilizer; chlorophyll content; *Triticum aestivum*; yield attributes; analysis of variance.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is among the oldest domesticated cereals and remains a principal source of dietary energy and protein for a large share of the global population. India is the second-largest producer of wheat in the world after China; national output reached a record of about 112 million tonnes in 2023-24 from roughly 31.8 million hectares, yet average productivity (about 3.5 t ha⁻¹) still trails that of intensively managed systems in Europe and China. Closing this yield gap without compounding the environmental costs of chemical fertilization has therefore become a central objective of cereal agronomy.

The intensive use of synthetic nitrogen and phosphorus fertilizers, although effective in raising yields, is associated with soil degradation, nutrient leaching, greenhouse-gas emission and rising input costs. Biofertilizers and organic bio-stimulants are increasingly promoted as eco-friendly and economical complements to mineral fertilizers because they require lower energy inputs while improving soil biological fertility and crop stress tolerance (Basu *et al.*, 2021; Wahab *et al.*, 2025). Plant growth-promoting rhizobacteria (PGPR) and related bio-inputs enhance plant performance through several direct mechanisms, including biological nitrogen fixation, phosphate solubilization, siderophore production and the synthesis of phytohormones such as auxins, gibberellins and cytokinins (Kalam *et al.*, 2020; Swarnalakshmi *et al.*, 2020).

A growing body of recent field evidence confirms that organic and microbial inputs improve wheat growth and grain quality. Seed inoculation with mycorrhizae and *Azotobacter* combined with humic acid significantly increased plant height, spike fertility, grain yield and chlorophyll content of wheat (Lamlom *et al.*, 2023), and the integration of compost with PGPR raised soil organic matter, nutrient availability and chlorophyll

while reducing dependence on chemical fertilizers (El-Akhdar *et al.*, 2024). Commercial microbial consortia have likewise enhanced tiller number, grains spike⁻¹ and grain yield when used to partially replace mineral NPK (Trivedi *et al.*, 2024). Because nitrogen is a structural constituent of the chlorophyll molecule and of Rubisco, improved nitrogen nutrition through organic inputs is frequently expressed as higher leaf chlorophyll and photosynthetic capacity (Kubar *et al.*, 2022; Oner, 2024).

Despite this progress, comparative information on the performance of distinct commercial organic products across a panel of wheat genotypes remains limited (Ghedabna *et al.*, 2023). The present study was therefore conducted to compare two such products - Bio-Elixir and APSA-80 - and to quantify, with formal analysis of variance, their effects on the growth, yield attributes and chlorophyll content of six widely grown wheat genotypes.

MATERIALS AND METHODS

Experimental site and design

The field experiment was carried out at the experimental farm of Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India. The experiment was arranged as a two-factor (genotype x fertilizer) study using a randomized block layout with an individual plot size of 1 m². The two experimental factors were six wheat genotypes and three fertilizer regimes (unfertilized control, Bio-Elixir and APSA-80).

Plant material and treatments

Six bread-wheat genotypes - K-7903, HUW-234, UP-2425, Raj-3765, K-9644 and PBW-373 - were sown with a seed drill on 25 November and harvested the following 8 May. The first irrigation was given 20 days after sowing and subsequent irrigations were scheduled according to crop and soil

requirement. Each genotype was grown under three treatments: T0, unfertilized control; T1, Bio-Elixir; and T2, APSA-80. Bio-Elixir Agro is a bio-stimulant produced from freshly mined sapropel and peat; the sapropel fraction supplies natural vitamins, minerals and humus-forming microorganisms that activate soil metabolic processes, while the peat fraction improves soil structure, aeration and moisture-holding capacity. APSA-80 is an all-purpose spray adjuvant containing about 80 % active ingredients that improves the efficacy of agro-inputs and crop protection. To eliminate border effects, the outermost rows and a 20 cm strip from the upper and lower margins of each plot were discarded, and five competitive plants per plot were randomly selected for recording observations.

Growth and yield observations

At the reproductive stage, plant height was measured from the soil surface to the apex of the main stem, and the number of tillers per plant and spike length were recorded on the five selected plants and averaged. Grains per spike were counted on the same plants, and grain yield per plant was determined after separate threshing of the five plants and expressed on a per-plant basis.

Estimation of chlorophyll content

Chlorophyll a, chlorophyll b and total chlorophyll were estimated following the dimethyl sulfoxide (DMSO) method of Hiscox and Israelstam (1979). Fully expanded upper leaves were cut into small pieces, and a known weight (100 mg) was incubated with 7 mL DMSO at 65 °C for 50 min. The extract was made up to 10 mL with DMSO, and absorbance was read at 645 and 663 nm in a spectrophotometer against a DMSO blank. Pigment concentrations were calculated using the standard equations and expressed as mg g⁻¹ fresh weight:

$$\text{Chlorophyll a} = [12.7 (\text{A663}) - 2.69 (\text{A645})] \times V / (1000 \times W)$$

$$\text{Chlorophyll b} = [22.9 (\text{A645}) - 4.68 (\text{A663})] \times V / (1000 \times W)$$

$$\text{Total chlorophyll} = [20.2 (\text{A645}) + 8.02 (\text{A663})] \times V / (1000 \times W)$$

where A645 and A663 are the absorbances at 645 and 663 nm, V is the final extract volume (mL) and W is the fresh weight of the sample (g).

Statistical analysis

All traits were subjected to a two-factor analysis of variance (ANOVA) appropriate to the genotype x fertilizer structure. The genotype x fertilizer interaction mean square (10 degrees of freedom) served as the error term. Treatment and genotype means were tested with the F-statistic, and significant means were separated using the critical difference (CD) computed as $CD = SEd \times t_{0.05}$, where SEd is the standard error of the difference. The standard error of the mean ($SEm \pm$) and the coefficient of variation (CV %) were calculated for each trait. Effects were considered significant at $p = 0.05$; * and ** denote significance at $p = 0.05$ and $p = 0.01$, respectively, and NS denotes non-significance.

RESULTS

Growth and yield attributes

The analysis of variance revealed highly significant differences among the fertilizer treatments for plant height, spike length, grains spike⁻¹ and tillers plant⁻¹, and highly significant genotypic effects for every trait (Table 1, Table 3). Bio-Elixir (T1) consistently recorded the highest treatment means. Plant height increased from 93.13 cm in the control to 95.77 cm under Bio-Elixir ($F = 16.68$; $CD = 1.02$), and spike length rose from 9.37 to 10.12 cm ($F = 20.20$; $CD = 0.27$). Grains spike⁻¹ improved from 53.23 in the control to 57.90 under Bio-Elixir ($F = 24.11$; $CD = 1.59$), an

increase of about 8.8 %, while tillers plant-1 rose from 6.70 to 7.58 ($F = 16.42$; $CD = 0.35$). APSA-80 (T2) gave intermediate values that were generally close to, and often not significantly different from, the control.

Grain yield plant-1 followed the same numerical ranking (Bio-Elixir 11.98 g > APSA-80 11.50 g > control 11.27 g); however, the effect of fertilizer treatment on grain yield was not statistically significant ($F = 3.33$; $p =$

0.078), even though genotypic variation in yield was highly significant ($F = 18.28$). The low coefficients of variation for the agronomic traits (0.84-4.24 %) indicate good experimental precision. Among genotypes, the tall PBW-373 and HUW-234 had the greatest plant height, UP-2425 and K-9644 carried the most grains spike-1, and K-9644 and K-7903 combined high grain yield with strong responsiveness to Bio-Elixir (Figure 2).

Table 1. Effect of organic fertilizers on plant height, spike length, grains spike-1, tillers plant-1 and grain yield plant-1 of six wheat genotypes. C = unfertilized control; T1 = Bio-Elixir; T2 = APSA-80.

Genotype	Plant height (cm)			Spike length (cm)			Grains spike-1			Tillers plant-1			Grain yield plant-1 (g)		
	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2
K-7903	86.4	89.5	88.5	9.4	10.2	9.5	52.0	58.2	55.0	6.4	7.2	6.3	12.7	13.2	12.5
HUW-234	100.4	105.2	102.3	8.8	9.6	9.2	46.0	48.1	45.0	6.2	6.6	6.5	9.3	10.4	9.8
UP-2425	83.6	85.3	84.3	10.4	10.6	10.5	68.5	70.3	68.5	5.6	6.8	6.2	11.5	10.5	10.5
Raj-3765	89.6	91.0	91.2	9.2	9.8	9.8	47.0	52.2	48.0	7.8	9.0	8.3	11.4	12.6	11.8
K-9644	93.4	96.4	94.2	10.2	11.3	10.5	61.4	68.4	62.0	6.8	8.2	7.0	12.2	13.7	13.0
PBW-373	105.4	107.2	105.0	8.2	9.2	8.5	44.5	50.2	46.4	7.4	7.7	7.5	10.5	11.5	11.4
Mean	93.1	95.8	94.2	9.4	10.1	9.7	53.2	57.9	54.2	6.7	7.6	7.0	11.3	12.0	11.5
SEm ($\pm$)	0.30	0.32	0.31	0.06	0.08	0.07	0.49	0.51	0.50	0.09	0.11	0.10	0.18	0.20	0.19
CD ($p = 0.05$)	1.0	1.02	1.01	0.26	0.27	0.26	1.56	1.59	1.58	0.33	0.35	0.34	0.61	0.63	0.62
CV (%)	0.82	0.84	0.83	2.09	2.12	2.10	2.22	2.24	2.23	3.85	3.87	3.86	4.22	4.24	4.23
F (treatment)	16.58 **	16.68 **	16.62 **	20.18 **	20.20 **	20.19 **	24.09 **	24.11 **	24.10 **	16.40 **	16.42 **	16.4 1**	3.31 NS	3.33 NS	3.32 NS

SEm ($\pm$), CD ($p = 0.05$), CV (%) and F-values refer to the comparison among fertilizer-treatment means for each trait. ** significant at $p = 0.01$; NS = non-significant. Values are means of five plants per plot.

Chlorophyll content

Organic-fertilizer application significantly influenced leaf pigment composition (Table 2, Figure 1). Total chlorophyll increased from 1.957 mg g⁻¹ in the control to 2.050 mg g⁻¹ under Bio-Elixir ($F = 8.92$; $CD = 0.049$), and chlorophyll b showed the strongest treatment response, rising from 0.376 to 0.473 mg g⁻¹ - an increase of about 26 % ($F = 11.52$; $CD = 0.045$). In contrast, chlorophyll a was statistically unaffected by treatment ($F = 0.15$; NS), indicating that the gain in total chlorophyll was driven mainly by chlorophyll b. Genotypic differences were significant for all three pigments, with HUW-234 and PBW-373 maintaining the highest total chlorophyll across treatments.

Table 2. Effect of organic fertilizers on chlorophyll a, chlorophyll b and total chlorophyll content (mg g⁻¹ fresh weight) of six wheat genotypes.

Genotype	Chlorophyll a (mg g ⁻¹)			Chlorophyll b (mg g ⁻¹)			Total chlorophyll (mg g ⁻¹)		
	C	T1	T2	C	T1	T2	C	T1	T2
K-7903	1.609	1.620	1.612	0.348	0.368	0.357	1.96	1.99	1.97
HUW-234	1.616	1.738	1.675	0.457	0.563	0.521	2.08	2.31	2.21
UP-2425	1.486	1.554	1.538	0.321	0.364	0.349	1.82	1.92	1.89
Raj-3765	1.623	1.512	1.553	0.357	0.531	0.426	1.98	2.06	1.99
K-9644	1.501	1.446	1.469	0.348	0.425	0.379	1.85	1.88	1.85

PBW-373	1.618	1.542	1.514	0.426	0.585	0.569	2.05	2.14	2.08
Mean	1.575	1.569	1.560	0.376	0.473	0.433	1.96	2.05	2.00
SEm ($\pm$)	0.021	0.020	0.021	0.015	0.014	0.013	0.017	0.016	0.018
CD ($p = 0.05$)	0.065	0.062	0.063	0.041	0.044	0.046	0.048	0.049	0.050
CV (%)	3.07	3.05	3.06	8.20	8.19	8.19	1.93	1.92	1.91
F (treatment)	0.15 NS	0.15 NS	0.15 NS	11.53**	11.52**	11.51**	8.91**	8.92**	8.92**

SEm ($\pm$), CD ($p = 0.05$), CV (%) and F-values refer to the comparison among fertilizer-treatment means for each pigment. ** significant at $p = 0.01$; NS = non-significant.

Table 3. Summary of analysis of variance: treatment means, F-values for fertilizer and genotype effects, and precision statistics for all measured traits

Trait	Control	Bio-Elixir (T1)	APSA-80 (T2)	F (Treat.)	F (Var.)	SEm ($\pm$)	CD (0.05)	CV (%)
Plant height (cm)	93.13	95.77	94.25	16.68**	337.13**	0.32	1.02	0.84
Spike length (cm)	9.37	10.12	9.67	20.20**	42.10**	0.08	0.27	2.12
Grains spike-1	53.23	57.90	54.15	24.11**	178.19**	0.50	1.59	2.24
Tillers plant-1	6.70	7.58	6.97	16.42**	26.54**	0.11	0.35	3.87
Grain yield plant-1 (g)	11.27	11.98	11.50	3.33 NS	18.28**	0.20	0.63	4.24
Chlorophyll a (mg g ⁻¹)	1.576	1.569	1.560	0.15 NS	6.52*	0.020	0.062	3.05
Chlorophyll b (mg g ⁻¹)	0.376	0.473	0.433	11.52**	15.16**	0.014	0.045	8.19
Total chlorophyll (mg g ⁻¹)	1.957	2.050	1.998	8.92**	34.17**	0.016	0.049	1.92

F (Treat.) and F (Var.) are the variance ratios for the fertilizer and genotype effects, respectively. * $p = 0.05$; ** $p = 0.01$; NS = non-significant.

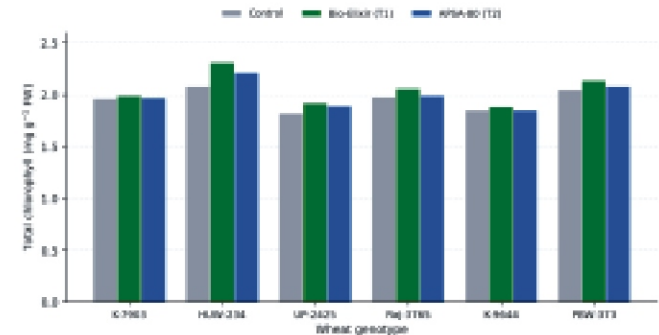


Figure 1. Total chlorophyll content (mg g⁻¹ fresh weight) of six wheat genotypes under the control, Bio-Elixir (T1) and APSA-80 (T2) treatments.

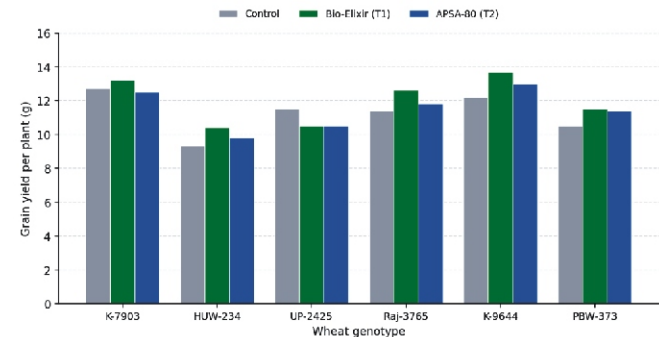


Figure 2. Grain yield per plant (g) of six wheat genotypes under the control, Bio-Elixir (T1) and APSA-80 (T2) treatments.

DISCUSSION

The results demonstrate that the sapropel- and peat-based product Bio-Elixir was the more effective of the two organic inputs, significantly enhancing vegetative growth, spike fertility and leaf chlorophyll relative to both the control and APSA-80. These responses are consistent with the recognized modes of action of organic bio-inputs, which improve nutrient availability and supply growth-regulating substances such as auxins, gibberellins and cytokinins, thereby stimulating root and shoot development (Basu *et al.*, 2021; Kalam *et al.*, 2020; Swarnalakshmi *et al.*, 2020). The humus-forming and moisture-conserving properties of the sapropel-peat matrix would be expected to improve the rhizosphere environment in a manner comparable to compost-based amendments, which have been shown to raise soil organic matter, nutrient supply and wheat productivity (El-Akhdar *et al.*, 2024).

The marked increase in chlorophyll b and total chlorophyll under Bio-Elixir agrees with the well-established role of improved nitrogen nutrition in pigment biosynthesis, given that nitrogen is a structural component of the chlorophyll molecule (Kubar *et al.*, 2022; Oner, 2024). The comparatively stable chlorophyll a, with a pronounced gain confined to chlorophyll b, suggests an adjustment of the light-harvesting antenna rather than of the reaction-centre pigment, a response also reported under organic and bio-fertilization of wheat (Lamlom *et al.*, 2023; García-López *et al.*, 2024). The intermediate and largely non-significant performance of APSA-80 is consistent with its primary function as a spray adjuvant rather than a nutrient source.

An important and rigorously demonstrated outcome of the present analysis is that, although Bio-Elixir numerically increased grain yield per plant, this effect was not statistically significant ($p = 0.078$). This pattern echoes field and meta-analytic evidence that bio-inputs reliably enhance growth and physiological vigour but translate into only modest, and sometimes statistically inconsistent, grain-yield gains within a single season (Ghedabna *et al.*, 2023; Trivedi *et al.*, 2024). The highly significant genotypic variation recorded for every trait further underscores that cultivar choice exerts a stronger influence on yield than the organic input itself, and that the benefit of such products is likely to be genotype-dependent and to accrue more clearly over multiple seasons through cumulative improvement of soil biological fertility (Wahab *et al.*, 2025).

CONCLUSION

Under a well-irrigated system, application of organic fertilizers improved the growth, spike fertility and chlorophyll content of wheat, with Bio-Elixir proving superior to APSA-80. Bio-Elixir significantly increased

plant height, spike length, grains spike⁻¹, tillers plant⁻¹, chlorophyll b and total chlorophyll, whereas its positive effect on grain yield per plant did not attain statistical significance in this single-season trial. Genotypic differences were highly significant for all traits, indicating that the response to organic inputs is cultivar-specific. Bio-Elixir can therefore be recommended as an effective organic bio-input for enhancing the vegetative vigour and photosynthetic capacity of wheat, and multi-season, replicated trials are warranted to confirm its longer-term contribution to grain yield and soil health within sustainable wheat-production systems.

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Received : April 2026; Revised : May 2026; Accepted : June 2026



COST–BENEFIT ANALYSIS OF ECO-FRIENDLY NUTRIENT SOURCES IN CHRYSANTHEMUM (*CHRYSANTHEMUM MORIFOLIUM* RAMAT.) CULTIVATION

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Abstract

The agronomic performance and economic viability of eco-friendly nutrient sources compared to conventional chemical fertilization in chrysanthemum (*Chrysanthemum morifolium* Ramat.) was evaluated in a one-year field experiment (2024-2025) at the agriculture farm of the Department of Agriculture, Swami Vivekanand Subharti University, Meerut. Nine treatments of nutrient management consisting of the recommended dose of fertilizer (RDF), farmyard manure, vermicompost, microbial biofertilizers such as *Azotobacter*, phosphate- and potassium-solubilising bacteria, fermented liquid organics known as jeevamrutha and panchagavya and their integrated combinations were implemented in a randomised block design with three replications. The evaluated parameters included growth, flowering, flower yield, post-harvest soil health and a complete cost and benefit analysis (cost-of-cultivation, gross and net returns, and benefit-to-cost ratio). The highest flower yield (134 q ha⁻¹), net returns (₹4,38,200 ha⁻¹) and benefit-to-cost ratio (3.14) were produced by T₆, which was the integration of 50% RDF and vermicompost (5 t ha⁻¹) and biofertilizer consortium, followed by T₉ which was the reduced-chemical integrated treatment (B:C 3.05). T₈ (the fully organic package) increased Organic carbon and microbial activity in soil but had a higher cost of inputs; thus, it had a lower benefit-to-cost ratio of 2.59 in comparison to the integrated treatments. T₁ (chemical control) provided a profit (B:C 2.66) but degraded post-harvest soil indicators consistently. According to the findings, replacing some amount of mineral fertilizers with vermicompost or biofertilizers is an economically rational and ecologically sustainable nutrient management method. Additionally, you can balance profitability and soil health by using these alternatives.

Keywords: *Chrysanthemum morifolium*; vermicompost; biofertilizers; jeevamrutha; benefit–cost ratio; integrated nutrient management; soil health

INTRODUCTION

The chrysanthemum (*Chrysanthemum morifolium* Ramat) is often referred to as the “Queen of the East,” and is one of the most important commercial ornamental crops in the global cut-flower and loose-flower industries due to its wide range of shapes, colours, long vase life, and ongoing demand surrounding uses (religious, decorative, and medicinal) all year round. Heavy feeders with relatively long field times, chrysanthemums are highly responsive to nutrient supply, which places fertilizer management as one of the main factors affecting both flower output and producer profit (Jiang et al., 2024). Therefore, maximizing both balance and source of nutrient inputs is central to the economics of commercial chrysanthemum production. Historically, conventional production systems have depended largely on synthetic NPK fertilizers to increase yields. However, improper and over-reliant usage of mineral fertilizers have had an increasingly negative effect on the physical, chemical, and biological properties of soil; the carbon content of soil; the balance of nutrients; and the environment, through processes such as: nitrate leaching; greenhouse gas emissions (Sellami & Lavini, 2023; Sande et al., 2024). In addition, rising fertilizer prices have resulted in increasing levels of interest in sustainable nutrient sources, that are able to support production while avoiding harm to soil health, and reducing reliance on purchase input products.

There are multiple organic and biological inputs that can be used as partial or full replacements for mineral fertilizer. One of these options is vermicompost, which is a type of amendment that contains high levels of nutrients and supports crop growth and yield by providing both macro- and micronutrients, and also humic substances, and plant-growth-promoting hormones; it is considered an excellent amendment for

improving soil fertility, as well as enhancing the resilience of soil to both abiotic and biotic stress (Rehman et al., 2023). Recycling of organic residue using earthworms represents a means for aligning nutrient management with circular economy principles through converting wastes into agricultural inputs (Malal et al., 2024). Microbial biofertilizers, including free-living nitrogen-fixing microbes (*Azotobacter*) and phosphate- and potassium-solubilising bacteria, can mobilise nutrients that would not be available to plants, secrete growth regulators, and help promote the health of roots by suppressing root pathogens, providing an inexpensive and sustainable complement to organic manures (Chaudhary et al., 2022). Fermented liquid organic formulations such as jeevamrutha and panchagavya, made largely from products of cattle kept on farms, offer an inexpensive source for providing microbial and biostimulant inputs to crops by enhancing nutrient dynamics and performance (Das et al., 2023).

The use of reduced amounts of mineral fertilizer combined with vermicompost and biofertilizers can produce equal or greater yields than complete chemical fertilizer applications, as well as provide increased soil microbial activity and available nutrients, according to studies conducted on chrysanthemums and other crops (Pathania et al., 2024; Sande et al., 2024). However, just because the agronomic result is better than an alternative method, it does not necessarily mean that it will be more profitable: because bulky organic amendments such as vermicompost have a higher total cost (per unit of nutrients), the profitability of a particular strategy will be determined by both how much a grower will yield in response to applying a given treatment and how much it costs to apply that treatment (Zhang et al. 2023). For commercial growers, choosing which treatment/strategy to use is therefore

not only based on yield but also on the benefit-to-cost ratio and net return/acre for a given treatment/strategy used.

There is a large body of literature on organic and biological inputs' influence on growth and flowering of chrysanthemums; however, there are only a limited number of studies comparing eco-friendly sources of nutrients with an explicit economic framework that incorporates cost of inputs, yield responses, and post-harvest soil health. This investigation evaluated the effects of conventional, organic, biological, and integrated sources of nutrients on growth, flowering, and yield of chrysanthemum. It also quantified the effect of each source of nutrient on post-harvest soil health indicators and provided a comprehensive cost-benefit analysis to determine the most economically sound and ecologically sustainable strategy for commercial production.

MATERIALS AND METHODS

Experimental site and growing conditions

The field trial was conducted over one growing season (2024-2025) on the agriculture property at the Department of Agriculture, Swami Vivekanand Subharti University in Meerut, Uttar Pradesh, India, located at approximately 28.98 degrees North and 77.71 degrees East at a height of approximately 219 metres above mean sea level. The location could be classified as Subtropical Semi-arid Area (Upper Gangetic Plain) and is identified by hot summers, a rainy season influenced by monsoons, and cool winters which are ideal for growing chrysanthemums. The soil in the experimental area is a sandy clay loam with good drainage and has an almost neutral reaction ($\text{pH} = 7.1$), medium levels of organic carbon (0.46 %), and low-to-medium levels of plant nutrients (N, P, and K) consistent with soils found in this area for growing flowers.

Planting material and experimental design

Standard commercial loose-flower chrysanthemum cultivar cuttings, which were unitary and well-rooted, were transplanted 30 cm apart on raised beds with 30 cm between them. The layout of the experiment was a randomised block design (RBD) of nine treatments (or sources of nutrients) replicated three times (total of 27 plots, all the same size). All plots received identical cultural practices (irrigation two pinches, staking, weeding and integrated pest/disease control) so that any differences between treatments are attributable to nutrient source. Each treatment (source of nutrients) was applied to the same size experimental micro-plot (0m²), the data generated from the micro-plots was used to calculate per hectare yield and economic estimates at the field level.

Treatment details

A total of nine different nutrient management strategies were used in this experiment, including full chemical fertilisation, bulky organic fertiliser, microbial biofertiliser (such as azotobacter chroococcum) and fermented liquid organic fertiliser, and various combinations of each of these options. Fertiliser application as recommended nutrient application (RDN) was 150 kg N ha⁻¹ + 100kg P₂O₅ ha⁻¹ + 75 kg K₂O ha⁻¹. Both vermicompost and farmyard manure were applied to beds during the final preparation. Bio-fertilisers were applied as an inoculation using a combination of soil and seedling dip methods, using the following three bio-fertilisers: azotobacter chroococcum, phosphate-solubilising bacteria and potassium-solubilizing bacteria. Jeevamrutha and Panchgavya were then periodically applied as drench (twice every two weeks) and 3% foliar spray, respectively, following standard preparation methods (Das et al., 2023).

Table 1. Description of nutrient management treatments evaluated in chrysanthemum

Code	Nutrient source / combination	Category
T ₁	100% recommended dose of fertilizer (RDF; 150:100:75 kg NPK ha ⁻¹) - chemical control	Conventional
T ₂	Farmyard manure (FYM) @ 20 t ha ⁻¹	Bulky organic
T ₃	Vermicompost @ 7.5 t ha ⁻¹	Bulky organic
T ₄	Azotobacter + PSB + KSB (5 kg ha ⁻¹ each, soil + seedling dip)	Biofertilizer
T ₅	Vermicompost @ 5 t ha ⁻¹ + (Azotobacter + PSB + KSB)	Organic combination
T ₆	50% RDF + Vermicompost @ 5 t ha ⁻¹ + (Azotobacter + PSB + KSB)	Integrated (INM)
T ₇	Jeevamrutha (500 L ha ⁻¹ drench) + Panchagavya (3% foliar)	Liquid organic
T ₈	Vermicompost @ 5 t ha ⁻¹ + Jeevamrutha + Panchagavya + biofertilizers	Full organic package
T ₉	75% RDF + Vermicompost @ 2.5 t ha ⁻¹ + (Azotobacter + PSB + KSB)	Integrated (INM)

RDF = recommended dose of fertilizer; PSB = phosphate-solubilising bacteria; KSB = potassium-solubilising bacteria; FYM = farmyard manure.

Observations recorded

The purpose of documenting vegetative development (height of plant, number of primary branches and measurement of plant spread), flowering characteristics (days to first bloom), flower quality (size of bloom & shelf-life of cut blooms) and yield in the form of total harvest weight of loose-cut flowers throughout the entire growing season (measured in quintals per hectare), five plants from each plot were randomly marked. Composite soil samples (depth 0–15 cm) were taken from each plot at the conclusion of the growing season to measure parameters of soil organic matter (SOM), available Nitrogen (N), Phosphorus (P) and Potassium (K) in addition to dehydrogenase activity (DHA) and microbial biomass carbon (MBC) using established chemical assays as a means to evaluate the impact of each nutrient source on

soil health (Pathania et al., 2024).

Economic analysis

In each treatment, a complete economic analysis of partial budgeting was done. The costs of cultivation were computed using only variable costs including planting material, nutrients, irrigation, labour to apply chemicals and work in the field, plant protection, and harvesting all evaluated at their local market prices and extrapolated from the micro-plot level to a per hectare level. The gross returns were calculated by multiplying the marketable flower production (per hectare) by the average price received at farm gate (₹ 4,800 q⁻¹), point of sale (during the 2024-2025 season) per loose weighed chrysanthemum flower. The major indicators used in the calculation of economic indicators were as follows:

Net returns (₹ ha⁻¹) = *Gross returns* - *Cost of cultivation*

Benefit–cost (B:C) ratio = *Gross returns* ÷ *Cost of cultivation*

The benefit–cost ratio was used to assess how economically efficient various nutrient sources were because it represents the amount of return generated for every rupee invested, thereby helping growers make decisions (Zhang et al., 2023).

Statistical analysis

Analysis will be performed using an analysis of variance (ANOVA) for a randomized block design. Treatment means will be compared using the critical difference (CD) at the 5% probability level. The standard error of the mean (SEm±) will be reported for these comparisons. All analyses will be based on data collected in a single crop season (2024-2025).

RESULTS

Vegetative growth and flowering

Nutrient source significantly influenced all growth and flowering attributes (Table 2). The integrated treatment T₆ (50% RDF + vermicompost + biofertilizers) recorded the tallest plants (52.3 cm), the greatest number of branches (13.6 plant⁻¹), and the widest plant spread (38.9 cm), closely followed by the reduced-chemical integrated treatment T₉ and the full organic package T₈. The same treatments advanced flowering: T₆ reached first flowering earliest (69 days), whereas the bulky-organic and liquid-organic treatments applied alone (T₂ and T₇) were the slowest. Flower quality followed an identical pattern, with T₆ producing the largest flowers (6.5 cm diameter) and the longest shelf life (8.3 days). Treatments combining mineral fertilizer with organic and biological sources thus consistently outperformed both the chemical control and the sole-organic treatments.

Table 2. Effect of eco-friendly nutrient sources on growth, flowering, and flower quality of chrysanthemum (2024–25).

Treat ment	Plant height (cm)	Branc hes plant ⁻¹	Plant sprea d (cm)	Days to 1st flower ing	Flowe r diam. (cm)	Flowe rs plant ⁻¹	Shelf life (days)
T ₁	42.1	9.8	30.4	78	5.1	34.2	6.2
T ₂	38.6	8.2	27.1	84	4.6	28.1	5.4
T ₃	44.8	10.6	32.6	76	5.4	37	6.6
T ₄	40.2	9	29	81	4.9	31.3	5.9
T ₅	47.5	11.8	34.8	73	5.8	42.4	7.1
T ₆	52.3	13.6	38.9	69	6.5	51.2	8.3
T ₇	39.4	8.7	28.2	82	4.7	29.4	5.6
T ₈	49.8	12.7	36.7	71	6.1	46.1	7.7
T ₉	51.1	13.1	38	70	6.3	49	8
SEm	0.71	0.24	0.58	0.92	0.11	0.86	0.14
±							
CD	2.12	0.72	1.74	2.76	0.33	2.58	0.42
(0.05)							

Values are means of the 2024–25 cropping season. SEm± = standard error of mean; CD (0.05) = critical difference at 5%.

Flower yield and soil health

All indicators of growth and flowering attributes were heavily impacted by all sources of nutrients (Table 2). The integrated treatment T₆ (50% RDF + vermicompost + biofertilizers) produced the tallest plants (52.3 cm), the most branches on each plant (13.6 plant⁻¹), and the greatest overall plant spread (38.9 cm), with T₉ (reduced-chemical integrated) and T₈ (organic-complete) close behind. Both treatments advanced to flowering quicker than other treatments, with T₆ flowering first at 69 days after transplanting and T₂ (bulky-organic alone) and T₇ (liquid-organic alone) taking longer to reach flowering than T₆. Similar results were seen with flower quality, where T₆ produced the largest flowers (6.5 cm in diameter) and had the longest shelf life (8.3 days). All treatments that combined inorganic fertilizer with organic and biological sources performed consistently better than chemical controls and organic-only treatments.

Soil health indicators collected from post-harvest measurements showed a clear and large separation in the chemical control as compared to eco-friendly (Table 3). The chemical control consistently had the lowest values for soil organic carbon (0.42%), dehydrogenase activity and microbial biomass carbon; whereas vermicompost and liquid organics treatments performed considerably better for each of these measurements. The total organic input treatment T₈ had the highest SOC (0.74%) and available N (301 kg ha⁻¹) and microbial biomass carbon and enzyme activity, with integrated treatment T₆ being second in all categories. Thus, eco-friendly sources contributed to soil biological activity despite not having an advantage in biomass production over integrated sources.

Table 3. Effect of eco-friendly nutrient sources on flower yield and post-harvest soil health indicators of chrysanthemum (2024–25).

Treat ment	Flower yield (q ha ⁻¹)	SOC (%)	Avail. N (kg ha ⁻¹)	Avail. P (kg ha ⁻¹)	Avail. K (kg ha ⁻¹)	DHA*	MBC*
T ₁	101	0.42	248	21.4	268	2.18	142
T ₂	87	0.58	262	23.8	281	2.94	188
T ₃	109	0.66	278	25.6	296	3.41	214
T ₄	92	0.55	270	26.9	289	3.12	201
T ₅	117	0.69	289	28.3	308	3.66	232
T ₆	134	0.71	298	30.1	318	3.88	248
T ₇	90	0.57	266	24.2	284	3.05	196
T ₈	123	0.74	301	30.8	322	3.95	256
T ₉	126	0.68	294	29.4	314	3.74	239
SEm	1.6	0.012	3.1	0.42	3.4	0.06	4.2
±							
CD (0.05)	4.8	0.036	9.3	1.26	10.2	0.18	12.6

*DHA = dehydrogenase activity ($\mu\text{g TPF g}^{-1} \text{ soil } 24 \text{ h}^{-1}$); **MBC = microbial biomass carbon ($\mu\text{g g}^{-1} \text{ soil}$); SOC = soil organic carbon.

Cost–benefit analysis

According To Economic Analysis (Table 4), The Profitability Of Treatments Was Ranked Differently From Yield, Therefore, The Input Cost Is Very Important. Biofertilizer Only (T4) Had The Lowest Cost Of Cultivation (Rs. 1,68,000 Ha⁻¹) While Vermicompost (T3 And T8) High Cost (Rs. 2,28,000 Ha⁻¹).The Integrated Treatment (T₆) Provided A High Yield But Also Had Moderate Input Cost So It Provided The Greatest Net Income (₹ 4,38,200 Ha⁻¹) And Best Benefit-Cost Ratio (B:C = 3.14). The Reduced Chemical Integrated Treatment (T₉), Was The 2nd Best Treatment (B:C = 3.05), Indicative Of The Economic Optimality Of The Partial Substitution Of Mineral Fertilizer With Vermicompost And Biofertilizers.

In contrast, the bulky-organic treatments applied separately were the least productive of all the treatments: the sole vermicompost (T₃) returned a benefit cost ratio of 2.23, whereas the sole farmyard manure (T₂) returned a benefit cost ratio of only 2.16 because their input economics and yield economics were not able to offset their costs. In addition, the fully organic package T₈, which produced the best

agronomic and soil health results, had a benefit cost ratio of 2.59, which was below the benefit cost ratio of the chemical control (2.66) and much lower than the integrated treatments because the costs of the organic inputs were high. The low cost liquid -organic treatment T₇ had an intermediate benefit cost ratio of 2.48. Collectively, the combination of the analyses show that the greatest economic return is achieved by using not the greatest quantity of organic inputs but by using a balanced combination of reduced rates of chemical fertilizer with cost-effective eco-friendly sources.

Table 4. Cost–benefit analysis of eco-friendly nutrient sources in chrysanthemum cultivation (2024–25).

Trt	Cost of cultivation (Rs. ha ⁻¹)	Flower yield (q ha ⁻¹)	Gross returns (Rs. ha ⁻¹)	Net returns (Rs. ha ⁻¹)	B:C ratio
T ₁	1,82,000	101	4,84,800	3,02,800	2.66
T ₂	1,93,000	87	4,17,600	2,24,600	2.16
T ₃	2,35,000	109	5,23,200	2,88,200	2.23
T ₄	1,68,000	92	4,41,600	2,73,600	2.63
T ₅	2,12,000	117	5,61,600	3,49,600	2.65
T ₆	2,05,000	134	6,43,200	4,38,200	3.14
T ₇	1,74,000	90	4,32,000	2,58,000	2.48
T ₈	2,28,000	123	5,90,400	3,62,400	2.59
T ₉	1,98,000	126	6,04,800	4,06,800	3.05

Gross returns computed at an average farm-gate price of Rs. 4,800 q⁻¹ of loose flowers. B:C ratio = gross returns ÷ cost of cultivation. All values are expressed on a one-hectare basis, scaled from micro-plot data. Figures in Indian Rupees (Rs.).

DISCUSSION

Results demonstrate that combining eco-friendly nutrient sources with a lower amount of mineral fertilizer can increase productivity, profitability, and sustainability of chrysanthemum production. For example, the most successful treatment (T₉) produced greater growth, flowering, yield, and economic returns compared to all other treatments, which is consistent with other studies showing a synergistic effect of organic amendments, microbial inoculants, and mineral fertilizers on chrysanthemum and other floriculture crops (Pathania et al., 2024; Sande et al., 2024). Vermicompost provides a slow-release, balanced source of macro- and micronutrients

in addition to humic substances and hormones that stimulate plant growth, whereas biofertilizers enhance the bioavailability of N, P, and K by fixing and solubilizing them; whereas residual mineral fertilizers provide sufficient nutrients for establishment during a critical period (Rehman et al., 2023; Chaudhary et al., 2022).

The significant increase in the postharvest soil organic carbon content, microbial biomass and enzyme activity in the vermicomposted or liquid organic planted plots demonstrates that these amendments are effective at restoring soil biological function, as has already been well documented, while at the same time reversing degradation due to the exclusive use of chemical fertilizers (Sellami & Lavini 2023; Rehman et al. 2023). The implementation of a circular economy model through the recycling of organic waste into vermicompost reduces the environmental burden of producing and disposing of both organic waste and synthetic fertilizers (Malal et al., 2024). The biostimulation and microbial contributions provided by jeevamrutha and panchagavya have also enhanced the microbial pool in the soil as well supported by many researchers who indicate that they contribute positively to nutrient dynamics and crop production (Das et al., 2023).

One of the major findings from this research is the gap between the optimal agronomic and economic conditions. For instance, even though T₆ produced the best soil health conditions, as well as a high quantity of flowers, the economic, or cost/benefit, ratio was comparatively low because the cost of organic inputs was very high. This finding is consistent with other research that has shown that the profitability of the use of organic amendments depends both on yield response and the cost of input rather than solely on yield (Zhang et al., 2023). The integrated treatments in the experiment performed best economically primarily

because they provided the greatest combination of benefits with the least amount of input costs associated with eco-friendly sources of inputs. Therefore, for commercial growers with limited resources, the use of partial substitution would be a more viable approach for adopting sustainable practices than total organic conversion (Sande et al., 2024).

There are some limitations in interpreting these results in terms of the economic rankings. Local prices for crops and compost can significantly influence the relative profitability of various treatment options—if compost can be produced locally at an affordable price and there is a premium for organically grown cut flowers, then the profitability of the organic treatments will likely improve. Longer-term multi-season studies should also demonstrate greater soil health and residual fertility advantages associated with environmentally friendly inputs, resulting in increased cumulative economic return for these inputs than was measured in a single season.

CONCLUSION

The best and most logical method to grow chrysanthemum flowers is to combine 50% of the recommended amounts of chemical fertilisers with vermicompost at 5 tonnes per hectare, and use a mixture of biofertilisers that contain *Azotobacter*, and both phosphate and potassium-solubilising bacteria. This agriculture practice produced the highest flower yields of 134 quintals per hectare, and produced the highest return on investment of Rs. 438,200/- per hectare, as well as a benefit-cost ratio of 3.14. Additionally, it improved soil health significantly more than chemical fertilisation alone when assessed after the harvest. Furthermore, while fully organic nutrition is beneficial for soil biological activity, it was not a viable economic alternative under existing input price

conditions, due to the cost of bulky organic materials. The conclusion of this research is that partially substituting chemical fertilisers with low-cost and environmentally-friendly sources can provide a nutrient-management recommendation for commercial chrysanthemum growers that is both profitable and sustainable. Additional research should be conducted to assess the long-term residual and price premium effects of these sources, and to expand the validity of this recommendation across different cultivars and agro-climatic conditions.

DECLARATIONS

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest: The authors declare that they have no competing interests.

Author contributions: All authors contributed to the conception and design of the study, data collection and analysis, and drafting and revision of the manuscript, and approved the final version.

Data availability: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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Received : January 2026; Revised : February 2026; Accepted : March 2026