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Effect of superphosphate, urea and bioinoculants on *Zinnia elegans* Jacq.

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Current agricultural practices depend heavily on chemical inputs, and their overuse seriously contaminates the soil health. Microbial bioinoculants are emerging as an effective greener replacement for chemical fertilizers. These bioinoculants are beneficial for plant growth and also diminishes pathogenicity. Here, we explored three microbial inoculants along with commonly used fertilizers, i.e., Superphosphate (S_P) and Urea (U_R) for their effectiveness on *Zinnia elegans* Jacq., that has considerable demand in the floral market. The experiment was conducted in three parts with recommended doses of fertilizers, low (half) doses, and high (double) doses in combination with *Glomus mosseae* (G_M), *Acaulospora laevis* (A_L) and *Pseudomonas fluorescens* (P_F), as microbial inoculants. The consortium of microbial inoculants (G_M+A_L+P_F) fed with the low dose of S_P and U_R gave the best results for growth parameters (Shoot and Root Length, Shoot and Root Weight), Floral traits (floral head number and diameter), mycorrhization pattern and for other physiological attributes (shoot phosphorus content, root phosphorus content, acidic phosphatase, alkaline phosphatase). Overall, the study establishes that microbial bioinoculants is a potential fertilizer supplement at the recommended dose supports optimum *Z. elegans* growth.

Keywords: Arbuscular mycorrhizal fungi (AMF), Chemical fertilizers, Floral yield, Flowering

The demand for ornamental flowers and flowering plants for cultural and aesthetic values is increasing day-by-day[1]. *Zinnia elegans* Jacq. (Fam. Asteraceae), commonly called zinnia, is among the most important annual summer flowers[2]. They have brilliant bright coloured flower heads (red, pink, orange, yellow and white) to attract butterflies and are suitable for bordering the landscape as they grow easily[3]. They are temperature tolerant and are useful in cottage gardens, rock gardens and pots[4]. The plant is splendid for cutting and can be used as cut flowers and/or bedding flowers due to their large range of diverse forms (single, semi-double and double), sizes and colours[4]. Application of superphosphate and Urea are important for getting macronutrients like phosphorous and nitrogen but in a controlled manner[5]. Usually, the soil contains 0.05% (w/w) of phosphorus of which only a small portion is bioavailable (rarely exceeding 10 µM) for plants, where it constitutes ~0.2% of total plant dry weight[6]. Although, the plants in semi-arid areas are devoid of phosphorus in the soil as it becomes difficult for the plant to absorb by the roots[7].

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Chemical fertilizers superphosphate and urea can be directly applied to the plants that contribute to phosphorus and nitrogen demands of the plants which has increased from 40.3 kilotonnes (kt) in 2011-2012 to 88 kt in the year 2015-16 and is still increasing per capita per year[8]. Moreover, it is also observed that phosphorous and nitrogen released from superphosphate and urea are not sufficient to regulate the healthy metabolism of the plant[8]. But growers still apply these fertilizers in much higher quantity[9]. Over the time, particles of applied chemical fertilizers remain entangled between the voids of soil particles that sometimes create a problem for plant growth and also create soil nutrient instability as well degrade the water body alongside[9].

For this, an alternative agro-biosystem has to be in place to mitigate the adverse effect of fertilizers. One such approach could be use of microbial inoculants, such as Arbuscular Mycorrhizal Fungi (AMF), Phosphate Solubilizing Bacteria (PSB) like *Pseudomonas fluorescens*, etc.[1]. These microbes play a prominent role in the rhizosphere and are ubiquitous, non-specific, and remarkably acquainted to diverse environments[10]. AMF that is widespread to over 80% of vascular plants, have a promising role in

increasing plant growth and flowering by increasing water and nutrient absorption, and by stimulating growth hormones[11].

Pseudomonas fluorescens can also be regarded as plant-growth-promoting bacteria (PSPB), which help mycorrhizal fungi to colonize plant cells significantly, thus also called as mycorrhizal helper bacteria. The bacteria promote growth for sustainable development by producing siderophores and stimulating various biotic activities including auxin production inside the host[12]. Different phytohormones levels, namely gibberellin and cytokinin also get modulated with the formation of ACC deaminase, which declines the ethylene synthesis, thereby, senescence of flowers can be prevented[13]. Co-culturing of *P. fluorescens* with mycorrhizal fungi is reportedly easier to inoculate and influence crop growth efficiently in the pot as well as field trials[14]. *Pseudomonas* and AMF together increase nutritional stature in plants, including phosphorus, nitrogen, zinc, sulphur, potassium, iron, etc., beneficial plant growth and metabolism[15].

Combinations of AMF and *P. fluorescens* are also helpful for improving soil status by managing the soil structure (porosity and aggregation) and its ecological interactions among diverse organism in the mycorrhizosphere[16,17]. Mycorrhization is a symbiotic process where there is an exchange of phosphorus and carbon take place from extraradical hyphae of AMF to plants, and AMF gets carbon from the plant only when phosphorus is supplied to the plant[18,19]. Therefore, in this study, we explored use of microbial inoculants in minimizing the chemical fertilizers portion, and thereby reducing the cost. This we tested on *Zinnia elegans*, that has a demand in floral market.

Materials and Methods

Experimental site

The experimentation was carried out during April to July 2018 under controlled conditions (Temperature: 25±2.5°C; Humidity: 68±18%) of polyhouse in the Department of Botany, Kurukshetra University, Kurukshetra, India. Plantlets of *Zinnia elegans* measuring around 10 cm were purchased from Rama nursery, New Delhi and were sterilized with 0.5% sodium hypochlorite for a few minutes. The plantlets were confirmed for authentication using the PlantID database (plant.id) and also by the Missouri botanical garden online database (https://www.missouribotanical garden.org).

Experimental setup

A loamy soil containing 75.7 sand, 9.8% silt and 11.1% clay with 4.8% organic matter, 19.8 mg potassium kg^{-1}, 15.6 mg phosphorus kg^{-1}, 0.24 mg nitrogen kg^{-1} and pH 7.2 was collected from the botanical garden of Department of Botany, Kurukshetra University (coordinates at: 29° 57.46' N, 76° 48.95' E) for the experiment[21]. The soil was air-dried and mixed with sand at the ratio of 3:1. The mixture was sieved through 2 mm autoclaved at 121°C and 15 psi for two consecutive days as the soil bacteria can divide rapidly by usiing the nutrients present in the soil. The sterilized soil-sand mixture was filled in 24.5 × 25.5 cm earthenware pots for experiment.

Experimental design

The experiment was conducted in a complete randomized block design (CRBD) with five replicates of each. The doses (low, medium and high) of S_P and U_R were 0.28, 0.56 &1.12 g/pot and 0.188, 0.375 & 0.75 g/pot, respectively. For giving microbial inoculation, AMF (*G. mosseae* and *A. laevis*) colonized roots of maize (having 75-80% infection) were chopped, and 10% (w/w) mycorrhizospheric soil (containing 870-890 spores) was added around the rhizosphere of *Z. elegans* plantlets in pots. As AMF are obligate symbiont, the inoculum production should be prepared using maize as the host, as described further. Treatment of *P. fluorescens* was given by simply dipping the root of *Zinnia* plantlets in broth culture, for 10 min. Plantlets were regularly watered by giving Hoagland's solution devoid KH_2PO_4, after each 13 days.

Mass production of microbial inoculum

Prior to experiment, the microbial inoculum was first multiplied. However, AMF are obligate symbiont and hencecan't be grown in laboratory artificial condition. Therefore, for mass production of *G. mosseae* and *A. laevis*, they were first collected and from the mycorrhizosphere of *Zinnia* plants growing in Botanical Garden of Kurukshetra University isolated by wet-sieving method of An *et al*.[22]. For isolation, 20 g soil was thoroughly mixed in water and sieved through 710 and 45 μm sieves. The sediments left in 45 μm sieves were collected in 50 mL centrifugation tubes, which were filled up by 48% sucrose solution and centrifugation was done for 5 min at 1750 rpm[23]. The floating debris was decanted-off, and this solution was again centrifuged through 45 μm sieve. Spores retained on 45 μm sieve were rinsed by

running water and collected on Whatman paper no. 4. The collected spores were microscopically identified, which was based on colour, size, spore walls count and wall ornamentation, using manuals of Schenck & Pérez[24]. The starter inoculums for *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Acaulospora laevis* Gerd. & Trappe was formed by 'Funnel technique' utilizing *Zea maize* as host for ninety days[25]. After this, starter inoculums were shifted to earthen funnels and then to large earthenware pots for mass production.

Starter inoculum of *P. fluorescens*, with batch no. MTCC No. 103, was brought from the CSIR-Institute of Microbial Technology (IMTECH), Chandigarh, India. It was then cultured, and mass multiplied utilizing 'nutrient broth medium' containing 3 gL^{-1} beef extract, peptone, and 5 gL^{-1} NaCl, which was then incubated in BOD for 32°C for 48 h to form a concentration of 1×10^9 mL^{-1} colonies.

Harvesting and data analysis

After 90 days of experiment, the plants were harvested and uprooted carefully without damaging roots. The shoot and root length were noted with the help of scale. Each floral head was counted, and their diameter was measured with a scale. Harvested plants were washed, and their fresh weights were noted with the help of weighing balance. After noted the fresh weight, the plants, and the roots were kept in the oven (Universal NSW-143) at 35°C overnight, for weighing their dry weights. Roots and rhizospheric soil were taken for assessing mycorrhizal infection and AMF no. by Philips & Hayman[26], and Giovannetti & Mosse[27], respectively. The roots were washed with water, then by KOH (10%) and stained using trypan blue (0.01%). The infection percentage was determined by utilizing formula- (number of root segments colonized/total number of root segments) × 100.

Physiological analysis for calculating phosphatase activity and phosphorus content was estimated by Tabatabai & Bremner's (1969)[28] and Jackson's (1973)[29] method, respectively. For phosphatase activity method, 1.0 g of root sample was washed and homogenized with ice-cold sodium acetate buffer (acidic phosphatase activity) at 0.1 M and pH 4, and sodium bicarbonate buffer (alkaline phosphatase activity) at 0.05 N with pH 10. They were then centrifuged separately at 10000 rpm for 15 min[28]. The supernatant was collected and assessed for phosphatase activity, whereas for phosphorus content, Jackson's vanadomolybdo phosphoric yellow colour method[29] was used.

Statistical analysis

Statistical analysis was performed with SPSS software (11.5 version)[30]. The differences among the treatments means of every treatment were subjected to analysis of variance (ANOVA); further, the level of significance was estimated with using Duncan's multiple range test (DMRT).

Results

Zinnia plants fertilized with S_P and U_R fortified with AMF, and *P. fluorescens* gave satisfactory results as postulated. It was observed that low dose (half the recommended) treatments of S_P and U_R amalgamated with $G_M+A_L+P_F$ (consortium), gave the most excellent results. The low dose treatments surpassed medium and high dose treatments, as noticed in Table 1. Tables 2 and 3 describe the effect of S_P with AMF and *P. fluorescens*, while Tables 4 and 5 represent U_R treatments with AMF and *P. fluorescens*. Tables 2 and 4 data show that S_P treatments were far better than U_R treatments in all respect. Speaking of S_P treatments, the low dose of S_P coupled with consortium treatment gave the best results for plant growth, physiological attributes, floral yield, and mycorrhization. Growth measurements like shoot height (37.5±1.17) and root length (17.24±1.5) were maximum in this treatment. Consequences, the shoot fresh (21.49±1.43) and dry (13.77±0.4) weights along with root fresh (6.38±1.08) and dry (3.9±0.51) weights, were the highest in this treatment. Low dose of treatment and $G_M+A_L+P_F$ collectively, gave the most significant results for floral yield (head no.

Table 1 — Detail of fertilizers treatments along with the microbial inoculums

Fertilizers	Fertilizers + Microbial Inoculum	Control
LD (SP; UR)	LD (SP; UR) + *Glomus mosseae* (GM)	(*–SP; –UR; –Microbial inoculum)
MD (SP; UR)	MD (SP; UR) + GM	
HD (SP; UR)	HD (SP; UR) + GM	
	LD (SP; UR) + *Acaulospora laevis* (AL)	
	MD (SP; UR) + AL	
	HD (SP; UR) + AL	
	LD (SP; UR) GM+AL+PF (*Pseudomonas fluorescence*)	
	MD (SP; UR) + GM+AL+PF	
	HD (SP; UR) + GM+AL+PF	

[*minus/devoid of; LD: Low Dose; MD: Medium Dose; HD: High Dose]

Table 2 — Effect of AM fungi and Super Phosphate (SP) on the growth of *Zinnia elegans* Jacq.

Doses (S_P g pot^{-1})	Treatments	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Head No.	Head diameter (cm)
Half Recommended (Low)	Control*	23.8±0.88^{f}‡	7.98±0.54^{h}	11.77±1.79^{ef}	3.89±0.55^{h}	1.73±0.32^{h}	0.68±0.16^{gh}	4±0^{h}	2.73±0.49^{i}
	S_P	29.5±1.55^{d}	11.34±1.1^{d}	13.01±1.06^{def}	4.59±0.32^{g}	3.18±0.87^{fg}	0.94±0.09^{fg}	5±0.7^{gh}	3.9±0.54^{g}
	S_P+G†	35±1.12^{b}	16.01±1.01^{ab}	18.03±1.16^{b}	7.77±0.22^{d}	5.16±1.26^{bc}	3.07±0.38^{b}	10±1.22^{c}	6.49±0.75^{ab}
	S_P+A††	34.3±1.56^{c}	14.1±1.29^{c}	16.11±1.16^{c}	5.99±0.52^{e}	4.76±1.53^{c}	1.95±0.56^{c}	8±1.73^{de}	6.13±0.94^{bc}
	S_P+GAP$_f^{\wedge}$	37.5±1.17^{a}	17.24±1.5^{a}	21.49±1.43^{a}	13.77±0.4^{a}	6.38±1.08^{a}	3.9±0.51^{a}	15±2.54^{a}	7.08±0.68^{a}
Recommended (Medium)	Control	23.9±0.91^{f}	7.89±0.41^{h}	11.47±2.15^{ef}	4.02±0.32^{h}	1.71±0.34^{h}	0.73±0.15^{gh}	4±0.7^{h}	2.66±0.37^{i}
	S_P	30.5±1.49^{d}	10.21±0.86^{de}	14.19±1.19^{cd}	5.29±0.23^{f}	4.19±0.28^{ef}	1.22±0.25^{ef}	6±1.22^{fg}	4.52±0.58^{ef}
	S_P+G	35.6±0.73^{b}	14.69±1.32^{bc}	18.05±0.92^{b}	7.41±0.19^{d}	4.67±1.33^{d}	2.05±0.23^{c}	13±1^{b}	6.09±0.75^{bc}
	S_P+A	34.2±1.18^{c}	14.02±1.39^{c}	15.95±1.49^{c}	5.44±0.26^{f}	4.3±0.13^{ef}	1.53±0.15^{de}	7±1.73^{ef}	5.49±0.67^{cd}
	S_P+GAP$_f$	35.7±1.62^{b}	15.64±1.32^{b}	20.29±1.48^{a}	13.02±0.3^{b}	5.87±0.72^{ab}	3.11±0.68^{b}	12±2.23^{b}	6.8±0.33^{ab}
	Control	23.7±0.91^{f}	8.01±0.48^{h}	11.59±1.99^{bef}	4.01±0.34^{h}	1.69±0.36^{h}	0.72±0.14^{gh}	4±0.7^{h}	2.76±0.37^{i}
Double Recommended (High)	S_P	19.6±1.41^{g}	6.45±0.46^{i}	8.89±1.25^{f}	2.86±0.48^{i}	1.35±0.21^{i}	0.34±0.13^{h}	2±0.7^{i}	1.32±0.6^{j}
	S_P+G	25.3±0.74^{ef}	8.36±1.08^{fg}	13.61±1.59^{cde}	4.76±0.39^{g}	3.19±0.73^{fg}	1.17±0.17^{fg}	7±1.58^{ef}	3.07±0.58^{h}
	S_P+A	24.4±1.62^{ef}	8.22±1.31^{bg}	12.65±1.04^{def}	4.51±0.33^{g}	2.86±0.64^{g}	0.89±0.1^{fg}	5±1.22	2.8±0.52^{h}
	S_P+GAP$_f$	27.1±1.1^{e}	9.69±1.27^{ef}	15.02±0.79^{cd}	9.13±0.35^{c}	5.35±0.64^{bc}	1.71±0.57^{c}	9±1^{cd}	5.12±1.1^{de}
$P \leq 0.05$		0.908	0.796	1.4	0.26	0.59	0.25	1.66	0.47
$F_{4,10}$	Treatment (Tt)	114.208	85.057	70.289	1191.523	54.317	103.32	93.246	75.412
	Parameter (Pt)	347.227	162.997	55.776	265.075	21.159	69.375	42.281	97.197
	Tt × Pt	22.238	12.319	4.523	35.986	2.809	9.553	5.877	8.425

[Control*: Untreated; G†: *Glomus mosseae*; A††: *Acaulospora laevis*; P$_f^{\wedge}$: *Pseudomonas fluorescence*. ‡respective values are mean of five replicates, ±Standard deviation; Values in columns followed by the same letter are not significantly different, $P \leq 0.05$: least significant difference test]

Table 3 — Effect of AM fungi and Super Phosphate (SP) on Physiological parameters and mycorrhization of *Zinnia elegans*.

Doses (S_P g pot^{-1})	Treatments	Phosphorous content (%)		Phosphatase activity (IU g^{-1} FW)		AM spore no. g^{-10} of soil	AM root colonization (%)
		Shoot	Root	Acidic	Alkaline		
Half Recommended (Low)	Control*	0.344±0.096^{i}‡	0.459±0.091^{i}	0.658±0.139^{f}	0.824±0.064^{h}	0±0^{i}	0±0^{h}
	S_P	0.403±0.184^{h}	0.505±0.119^{h}	0.762±0.104^{ef}	0.926±0.103^{gh}	0±0^{i}	0±0^{h}
	S_P+G†	1.893±0.429^{b}	2.152±0.159^{b}	2.078±0.154^{abc}	2.218±0.154^{abc}	78±5.87^{c}	68.2±5.31^{bc}
	S_P+A††	1.784±0.094^{bc}	2.031±0.347^{bc}	1.981±0.194^{bc}	2.054±0.153^{cde}	64±5.24^{e}	65.2±4.81^{cd}
	S_P+GAP$_f^{\wedge}$	2.271±0.171^{a}	2.554±0.45^{a}	2.233±0.168^{a}	2.341±0.167^{a}	92±4.94^{a}	77.6±2.3^{a}
Recommended (Medium)	Control	0.318±0.065^{i}	0.451±0.121^{i}	0.653±0.092^{f}	0.855±0.087^{h}	0±0^{i}	0±0^{h}
	S_P	0.643±0.109^{g}	0.667±0.089^{g}	0.985±0.208^{f}	1.131±0.101^{g}	0±0^{i}	0±0^{h}
	S_P+G	1.758±0.099^{bc}	1.846±0.114^{cd}	1.953±0.191^{bc}	2.108±0.175^{bc}	72±7.17^{d}	61.2±2.89^{d}
	S_P+A	1.621±0.101^{cd}	1.764±0.091^{d}	1.886±0.179^{cd}	1.937±0.124^{e}	59±7.28^{ef}	54.2±3.56^{e}
	S_P+GAP$_f$	1.944±0.102^{b}	2.139±0.109^{b}	2.156±0.122^{ab}	2.227±0.178^{ab}	85±5.56^{b}	69.8±5.4^{b}
Double Recommended (High)	Control	0.315±0.069^{i}	0.435±0.132^{i}	0.651±0.095^{f}	0.853±0.084^{h}	0±0^{i}	0±0^{h}
	S_P	0.135±0.069^{ij}	0.219±0.147^{j}	0.401±0.063^{g}	0.573±0.057^{i}	0±0^{i}	0±0^{h}
	S_P+G	1.205±0.113^{e}	1.439±0.089^{e}	1.731±0.166^{de}	1.696±0.129^{f}	46±2.73^{g}	39.4±5.85^{f}
	S_P+A	0.989±0.083^{f}	1.103±0.239^{f}	1.563±0.174^{e}	1.631±0.074^{f}	36±3.74^{h}	32.2±3.96^{g}
	S_P+GAP$_f$	1.482±0.075^{d}	1.643±0.111^{de}	1.912±0.084^{cd}	1.973±0.109^{de}	58±4.74^{f}	41.4±3.04^{f}
$P \leq 0.05$		0.11	0.14	0.105	0.09	3.76	2.43
$F_{4,10}$	Treatment (Tt)	343.784	252.238	326.775	405.047	1158.412	1324.715
	Parameter (Pt)	81.855	59.308	29.916	54.509	140.376	236.989
	Tt × Pt	8.077	6.242	3.363	5.759	23.741	40.173

[Control*: Untreated; G†: *Glomus mosseae*; A††: *Acaulospora laevis*; P$_f^{\wedge}$: *Pseudomonas fluorescence*. ‡respective values are mean of five replicates, ±Standard deviation; Values in columns followed by the same letter are not significantly different, $P \leq 0.05$: least significant difference test]

15±2.54; head diameter 7.08±0.68), total phosphorus content (root 2.554±0.45; shoot 2.271±0.171), total phosphatase activity (alkaline 2.341±0.167; acidic 2.233±0.168), and mycorrhization (AM no. 92±4.94; AM colonization 77.6±2.3). The least reading was noticed in high dose (double the recommended) treatments.

The low dose of U_R treatments together with G_M+A_L+P_F, proved to be the optimal treatment for growth, mycorrhization and yield. Shoot height (34.6±1.19) including its fresh (20.16±1.16) and dry (11.66±0.95) weight, root length (15.4±1.36) including its fresh (5.02±1.25) and dry (2.54±0.46) weight were recorded superior in this treatment. The low dose of U_R

Table 4 — Effect of AM fungi and Urea (UR) on the growth of *Zinnia elegans*

Doses (S_P g pot^{-1})	Treatments	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Head No.	Head diameter (cm)
Half Recommended (Low)	Control*	25.8±0.66^{e}‡	7.9±0.41^{def}	11.61±1.74^{e}	3.99±0.47^{g}	1.81±0.57^{ef}	0.71±0.06^{f}	4±0^{e}	2.73±0.49^{h}
	U_R	28.1±1.93^{d}	8.44±0.91^{de}	11.99±1.09^{de}	4.17±0.19^{fg}	2.98±0.98^{c}	0.85±0.19^{e}	5±1^{d}	3.33±0.58^{gh}
	U_R+G†	32.1±1.18^{bc}	14.49±0.68^{abc}	15.82±2.06^{b}	7.19±0.28^{d}	4.11±0.55^{ab}	1.53±0.39^{d}	7±0.7^{c}	6.18±0.63^{ab}
	U_R+A††	31.6±1.98^{bc}	13.6±1.95^{bc}	14.81±1.51^{bc}	5.41±0.27^{e}	4.43±1.59^{a}	1.93±0.44^{bc}	9±1.58^{b}	5.87±0.76^{bc}
	U_R+GAP$_f^{\wedge}$	34.6±1.19^{a}	15.4±1.36^{a}	20.16±1.16^{a}	11.66±0.95^{a}	5.02±1.25^{a}	2.54±0.46^{a}	13±1.58^{a}	7.05±0.68^{a}
Recommended (Medium)	Control	25.7±0.73^{e}	8.02±0.52^{def}	11.65±1.75^{e}	4.12±0.5^{g}	1.83±0.61^{ef}	0.71±0.06^{f}	4±0.7^{e}	2.64±0.4^{h}
	U_R	27.6±1.4^{d}	9.48±0.77^{d}	12.96±1.21^{cd}	4.65±0.21^{f}	3.19±0.45^{bc}	0.86±0.15^{e}	7±0.7^{c}	4.16±0.96^{fg}
	U_R+G	31.3±0.57^{c}	14.25±1.36^{abc}	15.16±1.51^{b}	7.37±0.17^{d}	3.92±0.21^{b}	1.32±0.2^{de}	10±1.22^{b}	5.73±0.33^{cd}
	U_R+A	30.7±1.12^{c}	13.04±1.79^{c}	14.41±1.12^{bc}	5.54±0.18^{e}	3.3±0.75^{bc}	1.01±0.34^{de}	10±1.58^{b}	5.35±0.45^{de}
	U_R+GAP$_f$	33.1±2.05^{ab}	14.68±1.67^{ab}	18.34±1.17^{a}	10.72±0.55^{b}	4.48±0.69^{a}	2.07±0.24^{b}	12±1.41^{a}	6.38±0.74^{ab}
Double Recommended (High)	Control	25.6±0.69^{e}	8.03±0.32^{def}	11.53±1.74^{e}	4.14±0.41^{g}	1.85±0.59^{ef}	0.72±0.06^{f}	3±1.22^{e}	2.75±0.37^{h}
	U_R	20.9±0.98^{g}	5.12±0.74^{g}	7.44±1.58^{f}	2.58±0.31^{h}	1.25±0.14^{g}	0.28±0.1^{g}	2±1^{f}	1.21±0.38^{i}
	U_R+G	25.8±0.27^{e}	7.26±1.07^{ef}	12.27±0.53^{d}	4.45±0.27^{fg}	3.27±0.76^{bc}	1.11±0.17^{de}	5±2^{d}	2.69±1.11^{hi}
	U_R+A	24.8±1.37^{f}	6.42±0.72^{fg}	11.64±1.75^{de}	4.13±0.09^{g}	2.61±0.29^{de}	0.84±0.13^{e}	5±0^{d}	2.22±0.71^{hi}
	U_R+GAP$_f$	25.9±0.75^{e}	8.21±1.31^{de}	14.23±0.84^{bc}	8.95±0.24^{c}	4.12±1.06^{ab}	1.59±0.43^{cd}	7±1.73^{c}	4.83±0.97^{ef}
$P \leq 0.05$		0.91	0.844	1.056	0.294	0.584	0.198	1	0.499
$F_{4,10}$	Treatment (Tt)	61.451	60.973	50.87	678.609	26.991	65.977	75.87	64.266
	Parameter (Pt)	161.891	151.664	24.988	137.086	9.935	31.215	78.478	86.863
	Tt × Pt	10.354	12.623	3.874	14.806	2.411	4.441	5.109	8.024

[Control*: Untreated; G†: *Glomus mosseae*; A††: *Acaulospora laevis*; $P_f^{\wedge}$: *Pseudomonas fluorescence*. ‡respective values are mean of five replicates, ±Standard deviation; Values in columns followed by the same letter are not significantly different, $P \leq 0.05$: least significant difference test]

Table 5 — Effect of AM fungi and Urea (UR) on Physiological parameters and mycorrhization of *Zinnia elegans*

Doses (g pot^{-1})	Treatments	Phosphorous content (%)		Phosphatase activity (IU g^{-1} FW)		AM spore no. g^{-10} of soil	AM root colonization (%)
		Shoot	Root	Acidic	Alkaline		
Half Recommended (Low)	Control*	0.272±0.045^{hi}‡	0.394±0.129^{g}	0.636±0.049^{f}	0.766±0.06^{i}	0±0^{h}	0±0^{f}
	U_R	0.383±0.098^{gh}	0.425±0.147^{f}	0.754±0.073^{ef}	0.945±0.107^{h}	0±0^{h}	0±0^{f}
	U_R+G†	1.814±0.132^{bc}	1.984±0.158^{b}	1.832±0.114^{ab}	1.927±0.116^{abc}	72±4.94^{c}	63.8±2.77^{b}
	U_R+A††	1.784±0.094^{bc}	1.851±0.138^{bc}	1.655±0.141^{c}	1.701±0.067^{def}	65±4.63^{d}	58.8±2.58^{c}
	U_R+GAP$_f^{\wedge}$	2.046±0.082^{a}	2.469±0.393^{a}	1.971±0.066^{a}	2.037±0.169^{a}	88±6.51^{a}	71.6±2.07^{a}
Recommended (Medium)	Control	0.229±0.045^{hi}	0.495±0.166^{g}	0.645±0.103^{f}	0.853±0.086^{i}	0±0^{h}	0±0^{f}
	U_R	0.512±0.082^{g}	0.559±0.102^{f}	1.123±0.149^{e}	1.286±0.167^{h}	0±0^{h}	0±0^{f}
	U_R+G	1.644±0.112^{cd}	1.719±0.154^{c}	1.702±0.109^{bc}	1.833±0.159^{bcd}	67±4.74^{d}	56.6±3.64^{c}
	U_R+A	1.526±0.096^{d}	1.658±0.079^{c}	1.566±0.082^{cd}	1.635±0.101^{ef}	56±2.91^{e}	51.2±5.54^{d}
	U_R+GAP$_f$	1.893±0.429^{ab}	2.012±0.086^{b}	1.837±0.128^{ab}	1.966±0.148^{ab}	78±6.2^{b}	65.4±3.04^{b}
Double Recommended (High)	Control	0.251±0.067^{hi}	0.415±0.233^{g}	0.643±0.106^{f}	0.859±0.093^{i}	0±0^{h}	0±0^{f}
	U_R	0.115±0.065^{i}	0.127±0.106^{h}	0.399±0.085^{g}	0.432±0.049^{j}	0±0^{h}	0±0^{f}
	U_R+G	0.828±0.144^{f}	1.086±0.089^{de}	1.111±0.117^{e}	1.561±0.073^{fg}	41±5.24^{f}	32.2±3.34^{f}
	U_R+A	0.716±0.103^{f}	1.009±0.068^{e}	0.984±0.204^{e}	1.422±0.254^{gh}	33±4.47^{g}	24±2.23^{g}
	U_R+GAP$_f$	1.078±0.084^{e}	1.248±0.211^{d}	1.465±0.107^{d}	1.774±0.137^{cde}	44±2.91^{f}	36.4±2.07^{e}
$P \leq 0.05$		0.105	0.124	0.908	0.095	2.759	1.881
$F_{4,10}$	Treatment (Tt)	318.471	260.807	274.741	234.84	1198.271	1821
	Parameter (Pt)	157.732	99.446	127.523	37.471	220.958	433.666
	Tt × Pt	16.492	11.149	13.244	10.038	39.504	72.892

[Control*: Untreated; G†: *Glomus mosseae*; A††: *Acaulospora laevis*; $P_f^{\wedge}$: *Pseudomonas fluorescence*. ‡respective values are mean of five replicates, ±Standard deviation; Values in columns followed by the same letter are not significantly different, $P \leq 0.05$: least significant difference test]

conjointly with the consortium was proved to be an efficient treatment for head no. (13±1.58), head diameter (7.05±0.68), AM no. (88±6.51), and AM colonization (71.6±2.07). Physiological attributes like phosphatase activity (acidic 1.971±0.066; alkaline 2.037±0.169) with root (2.469±0.393) and shoot (2.046±0.082) phosphorus content, were also maximal in this treatment.

Discussion

There are many pieces of evidence of using microbes, which have beneficial effect on soil fertility and plant growth for many crop plants that supports present findings. Microbial inoculation concurrently with mineral fertilizers, prominently help agricultural fields to reduce the impact of toxic fertilizers and

improve soil fertility, hence crop growth[14]. It is described that when plants were inoculated with AMF, increase nutrient availability, photosynthetic efficiency, respiration and plant metabolism[31]. This ought to be the reason why *Z. elegans* showed higher growth rate in our experiment. AMF and *P. fluorescens*, additionally ameliorate the uptake of K^+, $Ca^{2+,}$ and Mg^{2+} jointly with Pi (inorganic phosphate), that upsurge the plant growth in our experiment[1,32].

Lewandowski *et al*.[33], described using sustainable and low-maintenance, AMF while reducing the number of chemical mineral requirements, that supports our findings. Furthermore, it is reported that AMF and *P. fluorescens* to produce siderophores, protons, nucleic acids, hydroxyl ions, specific enzymes, and other organic acids[34]. AMF and PSB colonization increases phosphorus uptake by increasing the ammonium assimilation via NH^{4+}/H^+ antiporter, the protons released in soil will lowers the pH forming sulfuric and nitric acids, which solubilize Pi by actin upon amphiphilic fatty substances[35]. These might be the reason why the floral head number and its diameter in our study had increased. Hormones such as auxin and gibberellin got stimulated, which results in higher shoot higher and root length of the treated *Zinnia* plants[1,15].

Previously, several workers reported that using AMF and PSB can reduce the application of chemical fertilizers to the mentioned floral crops[36,37]. As deliberated by many workers' chemical fertilizers can be mixed with biofertilizers (organic waste, microbes, etc.), which decreases the quantity generally used for cultivation[38]. As it is discussed that AMF and PSB have an antagonistic effect on the pathogenic organisms, so the fungicides, herbicides and insecticides application also cut down[39].

Due to unmanaged techniques, poor skills, low-quality fertilizers, and improper drainage system make India's production to attain a lower rank in the world. At high doses of fertilizers, it has also been described that a high concentration of nitrogen and phosphorus fertilizers present in the soil inhibits the bacterial and mycorrhizal fungal activity[40]. In our experiment, it was noted that total phosphatase activity and phosphorus content had increased, which has been confirmed by Sato *et al*.[40] that exudates from AM fungi activated phosphatase enzyme. The magnitude of phosphorus absorption is directly related to this enzyme, resulting in more root and shoot phosphorus uptake[14]. There is compelling evidence where AMF can regulate phosphorus uptake, even where soil phosphorus is limited; this strongly connects with our findings[41].

Additionally, Bergkemper *et al*.[42], suggested that microbial inoculation can release hydrolytic enzymes (phosphomonoesterases, phosphodiesterases, β-glucosidase, phytases, phosphatases, etc.), which mineralize phosphate from cadmium and uranium salts. This strongly supports our findings of increased phosphatase activity and phosphorus content. Work done by Peine *et al*.[43] is also in accordance with our results, that using higher doses of fertilizers, growth of plants got affected. AMF is responsible for an additional phosphorus uptake with the help of mycorrhizal hyphae, having relatively thinner than the rational roots that create a downstream depletion zone for quick absorption of phosphorus from soil in the form of polyphosphates[19]. Therefore, sustainable strategies favorable for soil fertility, microbial stability, and plant health should be understood before applying inorganic minerals[13]. It is suggested that, if proper bio fertilization is done, the optimum quantity of chemical fertilizers can be reduced. It has already been demonstrated the potential use of AMF and bacteria in agricultural soil[14,20]. Accordingly, floriculture practices should also adopt this strategy for the cultivation and production of floral crops[40]. This favors flower yield as well as vase life, which is a significant phenomenon for cut flowers. The floricultural industry is booming in India. Zinnia flowers are facing exceptional demands both in the domestic as well in the global market. Zinnia with a free-flowering habit, ability to produce the marketable flowers in a short period, a large variety of engaging colours with different shapes and sizes, along with a good keeping quality has lured the flower growers and the consumers.

Conclusion

The results reveal that AMF and PSB are two beneficial microbial groups that could enhance the plant growth, improve the soil structure and fertility, and increase the yield. This approach is eco-friendly sustainable, easy-to-use, and economical. The AMF and PSB work in combination as they make the unavailable phosphorous easily accessible, and thereby the AMF hyphae absorb at ease. Our results recommend the right quantity of fertilizers and microbial combination with the adequate edaphic and

climatic condition as well as management practices to enhance crop productivity and soil fertility. This study demonstrates how overuse of chemicals affects the soil environment and plant health and suggests the importance of bio-inoculants for field as well as human health.

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Conflict of interest

Authors declare no conflict of interests.

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