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Research Article

Influence of Biostimulants on Important Traits of *Zinnia elegans* Jacq. under Open Field Conditions

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The efficacy of microbial treatment on growth, yield, and nutrient uptake is very well acknowledged for field crops. However, the use of microbes for *Zinnia elegans* has rarely been exploited under field trials. Therefore, in this study, we have evaluated the efficacy of different microbial bioinoculants on sixteen morphological and nine biochemical traits of *Z. elegans*. We used two arbuscular mycorrhizal fungi (AMF) (*Glomus mosseae* (G) and *Acaulospora laevis* (A)) along with *Trichoderma viride* (T) and *Pseudomonas florescence* (P) as five different treatments under open field conditions, in a randomized complete block design. There were significant differences for all of the traits studied. Treatment 5 (G + A + T + P) was noted as the best treatment for the improvement of morphological characters, whereas Treatment 4 (G + A + P) was most significant for the biochemical trait improvement in *Z. elegans*. Overall, this study provides useful insight into the bioinoculant treatment that can be applied to improve the yield and flower quality of *Z. elegans* under open field conditions.

1. Introduction

Zinnia elegans Jacq., a member of family Asteraceae, is an important summer garden flower with diverse shade of colours [1]. *Z. elegans* can accumulate heavy metals like cadmium in its shoots; therefore, it can be used as an eradication treatment for soils contaminated with heavy metals [2, 3]. *Z. elegans* can tolerate harsh environmental conditions, and its production on marginal lands during harsh summer is preferred under north Indian conditions [4]. In order to get above average production, the use of heavy dosages of chemical fertilizers is not uncommon. But, chemical fertilizers alter the soil nutrient profile and could result in water and air pollution [5]. Hence, there is a need to identify sustainable and eco-friendly practices for higher production, and the use of microbial inoculants is one of the commonly used sustainable approaches [6].

Microbes especially the arbuscular mycorrhizal fungi (AMF) has a significant influence on the terrestrial ecosystem where they perform a key role in providing ecosystem services and species richness [7, 8]. AMF is the obligate association of plant roots with beneficial fungi (belonging to either basidiomycetes and ascomycetes clades), and it is well recognized that vascularophytes get up to 86% of their nutrients and water requirements via mycorrhization, whereas their host plant supports fungal partner in its carbon requirement [9, 10]. This mycorrhizal association along with different kinds of soil microbiota like *Trichoderma viride* and *Pseudomonas fluorescens* acts as biostimulants, in order to significantly improve the uptake of even less mobile elements like phosphorous [11, 12]. Biostimulants treatment as compared to soil fertilizers improves floral traits as well as soil nutrient profile, even with its small quantities [13, 14].

Over a course of time, AMF has established a healthy relationship in several plant hosts, for example, *Pelargonium peltatum* showed an increased shoot phosphorous/potassium concentrations and flower numbers when inoculated by AMF culture [15]. Another study reported that microbial inoculation in hyacinth has resulted in the higher concentration of N, P, and K in hyacinth flower [16]. Likewise, *Pseudomonas fluorescens* is a Gram-negative

rhizobacterium well known for its role in plant development [17]. *Trichoderma viride* is popular for its use in floriculture practices, in order to obtain better plant growth by assisting the AMF species already present in the soil [18].

Pseudomonas fluorescence and *Trichoderma viride* work as the mycorrhizal helper, and these are well demonstrated to promote mycorrhizal development as well as to provide immunity against plant pathogens like *Pythium* sp. [19]. Several microbial treatments are known for their positive effect in different flowering plants [20–22]. However, the effect of AMF treatment alone or in combination with *Pseudomonas fluoresces* and *Trichoderma viride* on the roots of *Z. elegans* under open field condition has not been explored. Although, we have shown the effects of these microbes for *Zinnia elegans* under polyhouse conditions [11]. Nonetheless, the behaviour of microbial treatment under open field conditions is still unknown Therefore, the main objective of our study was to select the best and efficient microbial amalgamation among *Glomus mosseae* (*Funneliformis mosseae*), *Acaulospora laevis*, *Pseudomonas fluoresces*, and *Trichoderma viride* for *Z. elegans* under open field conditions.

2. Materials and Methods

2.1. Study Area and Experimental Design. The experiment was conducted at Botanical Garden of Kurukshetra University, Haryana, India. The site soil was sandy loam in texture with a pH of 8.2. However, the soil nutrients were determined as 0.35 mg nitrogen kg^{-1}, 1.68 mg phosphorous kg^{-1}, and 22.98 mg potassium kg^{-1} following the package and practices provided elsewhere [23]. Further, the indigenous density of AMF spores was 26 ± 5 spores 10^{-1} g soil which was counted by the gridline intersect method of Adholeya and Gaur [24], whereas the soil diversity of AMF species was assessed and the major constituents identified were *Glomus mosseae*, *G. convolutum*, *Acaulospora elegans*, *A. laevis*, and *Scutellospora calospora* using different manuals [25–27].

The experiment was laid out from April 2018 to July 2018 in a random complete block design (RCBD) with a total of six treatments grown across six separate flowerbeds (thoroughly ploughed) with ten plantlets per treatment. The plot size was 1×2 m, that was parted by a 0.25 m wide alleyway. In our previous work, a pot experiment was performed in polyhouse condition and five treatments were identified promising, i.e., *Glomus mosseae* + *Acaulospora laevis* (Treatment 1), *G. mosseae* + *Trichoderma viride* (Treatment 2), *G. mosseae* + *A. laevis* + *T. viride* (Treatment 3), *G. mosseae* + *A. laevis* + *Pseudomonas florescence* (Treatment 4), and *G. mosseae* + *A. laevis* + *T. viride* + *P. florescence* (Treatment 5). Hence, these treatments were selected for the open field trail along with the control (Treatment 0), to assess the best treatment for growers as these microbes are also naturally present in the soil [11]. Therefore, to validate the effect of bioinoculant formulation under open field conditions, this experiment was carried out using the treatments provided in Table 1.

Table 1: Set of treatments used for the inoculation of *Zinnia elegans*.

Treatment	Composition
Treatment 0 (T0)	Flowerbed with no extra inoculants (control)
Treatment 1 (T1)	*G. mosseae* + *A. laevis* (G + A)
Treatment 2 (T2)	*G. mosseae* + *T. viride* (G + T)
Treatment 3 (T3)	*G. mosseae* + *A. laevis* + *T. viride* (G + A + T)
Treatment 4 (T4)	*G. mosseae* + *A. laevis* + *P. fluorescens* (G + A + P)
Treatment 5 (T5)	*G. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* (G + A + T + P)

2.2. Plant Inoculation. For treatments (T1–T5), each plantlet was supplemented with uniform supply of AMF inoculum at the rate of 100 g/plant around the roots of plantlets containing 670–700 AMF spores counted by Adholeya and Gaur [24] method and at least 80–85% of AM infection assessed by "Rapid Clearing and Staining Method" of Phillips and Hayman [28]; this treatment was followed by immediate watering. Roots were dipped for 10 minutes for *P. florescence* treatment, that was cultured in nutrient broth medium. *Pseudomonas fluorescens* for the inoculation was applied at a concentration of 1×109 colony mL^{-1}. In case where *T. viride* treatment (density 3.4×10^8 CFU g^{-1} per treatment) was applied, CFU/g (colony-forming units per Gram) determination was based on the following formula: CFU/g = (no. of colonies × dilution factor)/weight of the culture plate.

2.3. Plant Characterization and Data Analysis. After 120 days, 6–7 plants were randomly selected and used for the measurement of traits. Morphological parameters were measured as root length (cm), shoot length (cm), and total leaf area (cm^2) at the harvest stage. Initial values of root fresh weight (g), shoot fresh weight (g), and leaf fresh weight (g) were used to determine the dry weight after over drying at 70°C. Whereas the number of floral heads and number of floret layers were measured at the full bloom stage. Floral heads were harvested at maturity for measuring the diameter and for fresh and dry weight. AMF diversity and AM spore quantification were noted from the rhizosphere of an uprooted plant, and a soil sample (~20 g) was analyzed based on the method defined elsewhere [29]. The AMF root colonization (%) was determined using the following formula: 100 × (number of root segments colonized/the total number of root segments).

Physiological parameters were analyzed as leaves samples were used to determine the total chlorophyll and carotenoid contents [30]. Anthocyanin content was estimated from the floral head using the method given by Tsushida and Suzuki [30]. Whereas root and shoot phosphatase activities were evaluated by the method of Tabatabai and Bremmer [31]. Whereas the phosphorus content was determined

based on "Jackson's vanadomolybdo phosphoric acid yellow colour method" [32].

Means of each treatment were exposed to analyses of variance (ANOVA) to detect differences among the six groups. The significance of differences among group means was evaluated using the least significant differences (LSD) $P < 0.05$ test. All these analyses were performed with the SPSS (11.5 version) software package [33]. The correlation coefficients (r) and their p values were estimated and plotted with the help of the package corrplot in the R environment [34].

3. Results

The analysis of variance (ANOVA) revealed the presence of highly significant differences (<0.001) among all of the six treatment groups. The result pertaining to growth, yield, and physiological parameters is illustrated in Tables 2 and 3.

3.1. Morphological, Mycorrhization, and Yield Parameters. In the experiment, morphological growth parameters, namely, plant length, root length, and shoot and root weight as well as yield was found increased over control plants (Table 2). Up to 76% increment was noticed for the growth when inoculated by biostimulants. Plant height was noticed prominent in case of T5 (56 ± 5.8) having *G. mosseae*, *A. laevis*, *T. viride*, and *P. fluorescens*, altogether followed by T4 (50 ± 3.4) having *G. mosseae*, *A. laevis* and *P. fluorescens* (Table 2). The same pattern was observed in the case of root length, where the longest was T5 (17.09 ± 1.35) followed by T4 (16 ± 1.3) (Table 2). Similarly, fresh shoot (17.25 ± 1.358) and root (11.757 ± 1.082) weight, as well as dry shoot (2.294 ± 0.489) and root (1.044 ± 0.345) weight were found highest in T5 followed by T4 (Table 2). Whereas the total leaf area was found largest in T4 (35.33 ± 0.054) followed by T5 (32.31 ± 0.03) (Table 2).

Though there was a slight difference in both the treatments, T4 proved to be the leading treatment for yield and biochemical attributes (Table 2). T4 was found with the extreme mycorrhizal infection (63 ± 3.87) and the largest number of AM spore (153 ± 13.4) followed by T5 with 58% (±6.68) mycorrhization and 140 (±10.3) AM spore count (Table 2). The floral head weight (fresh weight: 5.4 ± 0.469; dry weight: 1.66 ± 0.315; floral head number: 10 ± 1.15) was too noted maximum in T4 followed by T5 (fresh weight: 5.02 ± 0.312; dry weight: 1.17 ± 0.176; floral head number: 9 ± 1.63) (Table 2). Although the floral head diameter was measured largest in T5 (10.24 ± 0.665) followed by T4 (9.8 ± 0.421), the number of floret layers was maximum in T4 (7 ± 0.816) as compared to T5 (Table 2). The shoot length and root length changes across different treatments are represented in Table 2 and Figure 1. Root colonization (%) was around six times higher in the consortium treatment T5 (G + A + T + P) as compared to control (T0) (Figure 1). Interestingly, root lengths were almost similar in case of treatment T2 and T5 (Figure 1).

3.2. Plant Physiological Responses. The morphological outcomes of a plant are also determined by physiological and biochemical traits [35]. Plants with bioinoculants were observed with an improvement in all the physiological parameters considered for the experiment. Microbial colonization increased the total chlorophyll and carotenoid pigment content. Flower bed containing Treatment 4 (2.207 ± 0.236) was recorded with higher total chlorophyll and carotenoid content followed by T3 (1.797 ± 0.19) as compared to control T0 (0.059 ± 0.031) (Table 3). Similarly, anthocyanin content was found the maximum in the Treatment 4 (2.363 ± 0.285) followed by T3 (2.008 ± 0.121) (Table 3). Likewise, the phosphatase activity was at par in the T4 (acidic phosphatase: 0.708 ± 0.159; alkaline phosphatase: 0.961 ± 0.123). Correspondingly, phosphorous content was highest in the same treatment, i.e., T4 (root phosphorous: 3.746 ± 0.278; shoot phosphorous: 3.292 ± 0.197) (Table 3). Overall, for the biochemical traits, there were several fold differences among the treatments and T4 (G + A + P) was the best treatment for all of the biochemical traits (Table 3). The correlation analysis showed the presence of significant ($p < 0.05$) and positive correlation for all of the traits studied (Figure 2).

4. Discussion

In order to determine the microbial inoculant treatment, it is important to know the practicality and feasibility of microbes, especially of their combined formulation, as different microbes have a different degree of colonization and specificity [36]. As anticipated, we have observed a significant improvement with bioinoculants treatment in *Z. elegans* for important morphological and biochemical traits in *Zinnia elegans*. AMF is a well-known enhancer of water absorption along with macro and micronutrients uptake via extraradical hyphae emerging from infected their host plants root system [37]. This ought to be the reason for a significant increase in all important morphological traits of *Z. elegans* in our field study. Previously, with AMF treatment improvement for several morphological traits were recorded for other flower crops like Lilium, rose, and gazania [12, 38–40]. Likewise, an investigation carried out by Lazzara et al. [41] reported that inoculation with AMF noticeably increased the flower size and number, in Snapdragon and *Hypericum perforatum*.

AMF is a well-known regulator of plant auxins and gibberellins content and both are crucial for promotes flower development; this supports our finding of increased flower weight and diameter in *Zinnia elegans* [42]. Increased phytohormones especially the auxins stimulate the lateral roots formation owing to absorb more water and minerals [43]. A significant increase in total chlorophyll and carotenoid content was found to be increased which could be due to improved stomatal conductance [44]. Earlier, it was suggested that AMF treatment considerably improves the host growth by proving an important iron chelating low molecular weight "siderophores" which are beneficial for the photosynthesis and respiration in plants, ultimately enhancing the floral quantitative and qualitative characteristics [37, 45, 46].

Caser et al. [47] and Saini et al. [12] studied that AMF-inoculated plants showed an increase in secondary

Table 2: Effect of bioinoculants on morphological and floral parameters of *Zinnia elegans*.

Parameters	Treatment 0	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	*F* ratio	Probability
Shoot length (cm)	30 ± 3.5^{e}	40 ± 4.2^{c}	35 ± 2.1^{d}	41 ± 3.6^{c}	50 ± 3.4^{b}	56 ± 5.8^{a}	4.214	<0.001
Root length (cm)	4.64 ± 0.94^{e}	9.09 ± 1.24^{d}	14 ± 1.3^{b}	11.08 ± 1.15^{c}	16 ± 1.3^{a}	17.09 ± 1.35^{a}	1.332	<0.001
Fresh shoot weight (g)	9.3 ± 1.302^{c}	14.74 ± 1.178^{b}	13.81 ± 1^{b}	16.57 ± 1.616^{a}	16.93 ± 1.592^{a}	17.25 ± 1.358^{a}	1.473	<0.001
Dry shoot weight (g)	4.844 ± 0.933^{c}	9.655 ± 1.55^{b}	8.297 ± 0.996^{b}	11.558 ± 1.372^{a}	11.675 ± 1.741^{a}	11.757 ± 1.082^{a}	1.424	<0.001
Fresh root weight (g)	0.745 ± 0.054^{c}	2.05 ± 0.096^{a}	0.979 ± 0.104^{b}	2.109 ± 0.525^{a}	2.187 ± 0.53^{a}	2.294 ± 0.489^{a}	0.4	<0.001
Dry root weight (g)	0.207 ± 0.058^{c}	0.557 ± 0.037^{b}	0.452 ± 0.076^{b}	0.811 ± 0.107^{a}	0.985 ± 0.38^{a}	1.044 ± 0.345^{a}	0.236	<0.001
Leaf area (cm^2)	6.724 ± 0.224^{f}	23.38 ± 0.284^{d}	15.61 ± 0.22^{e}	27.72 ± 0.139^{c}	35.33 ± 0.054^{a}	32.312 ± 0.03^{b}	0.199	<0.001
Fresh leaf weight (g)	0.25 ± 0.047^{f}	0.535 ± 0.066^{d}	0.41 ± 0.04^{e}	0.634 ± 0.069^{c}	0.803 ± 0.092^{a}	0.727 ± 0.07^{b}	0.072	<0.001
Dry leaf weight (g)	0.05 ± 0.011^{d}	0.09 ± 0.005^{bc}	0.07 ± 0.006^{c}	0.097 ± 0.08^{b}	0.121 ± 0.025^{a}	0.108 ± 0.028^{ab}	0.018	<0.001
Root colonization (%)	22 ± 2.64^{f}	50 ± 3.91^{d}	35 ± 2.16^{e}	42 ± 4.54^{c}	63 ± 3.87^{a}	58 ± 6.68^{b}	4.585	<0.001
Spore number (per 10 g soil)	30 ± 5.8^{f}	100 ± 8.2^{d}	63 ± 6.1^{e}	81 ± 10.5^{c}	153 ± 13.4^{a}	140 ± 10.3^{b}	10.258	<0.001
Number of floral heads	2 ± 0.57^{d}	4 ± 1^{c}	4 ± 0.816^{c}	7 ± 1.29^{b}	10 ± 1.15^{a}	9 ± 1.63^{a}	1.225	<0.001
Floral head diameter (cm)	2.74 ± 0.639^{e}	7.61 ± 0.481^{c}	4.82 ± 0.495^{d}	9.62 ± 0.345^{b}	9.8 ± 0.421^{ab}	10.24 ± 0.665^{a}	0.564	<0.001
Floral head fresh weight	1.63 ± 0.253^{d}	4.53 ± 0.629^{b}	3.18 ± 0.404^{c}	4.06 ± 0.274^{b}	5.4 ± 0.469^{a}	5.02 ± 0.312^{a}	0.446	<0.001
Floral head dry weight (g)	0.33 ± 0.13^{e}	1.04 ± 0.197^{c}	0.7 ± 0.166^{d}	0.98 ± 0.608^{c}	1.66 ± 0.315^{a}	1.17 ± 0.176^{b}	0.338	<0.001
Number of floret layers	1 ± 0^{e}	5 ± 1.15^{c}	3 ± 1.29^{d}	5 ± 0.816^{b}	7 ± 0.816^{a}	2 ± 0.577^{d}	0.956	<0.001

*Means within rows separated by different letters are significantly different based on the least significant difference test (LSD).

Table 3: Effect of bioinoculants on biochemical and physiological attributes of *Zinnia elegans*.

Parameters	Treatment 0	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	*F* ratio	Probability
Chlorophyll *a* ($mg{\cdot}g^{-1}{\cdot}FW$)	0.556 ± 0.111^{d}	0.805 ± 0.173^{c}	0.954 ± 0.1^{c}	1.12 ± 0.122^{b}	1.419 ± 0.138^{a}	0.845 ± 0.143^{c}	0.1452	<0.001
Chlorophyll *b* ($mg{\cdot}g^{-1}{\cdot}FW$)	0.284 ± 0.096^{d}	0.461 ± 0.138^{c}	0.529 ± 0.145^{c}	0.677 ± 0.098^{b}	0.788 ± 0.112^{a}	0.514 ± 0.104^{c}	0.127	<0.001
Total chlorophyll ($mg{\cdot}g^{-1}{\cdot}FW$)	0.841 ± 0.177^{d}	1.266 ± 0.268^{c}	1.483 ± 0.179^{c}	1.797 ± 0.19^{b}	2.207 ± 0.236^{a}	1.359 ± 0.226^{c}	0.233	<0.001
Carotenoid ($mg{\cdot}g^{-1}{\cdot}FW$)	0.059 ± 0.031^{d}	0.213 ± 0.106^{bc}	0.126 ± 0.062^{c}	0.19 ± 0.112^{bc}	0.377 ± 0.118^{a}	0.301 ± 0.135^{ab}	0.109	<0.001
Anthocyanin (mg $100\,g^{-1}{\cdot}FW$)	0.992 ± 0.147^{d}	2.04 ± 0.248^{b}	1.372 ± 0.156^{c}	2.008 ± 0.121^{b}	2.363 ± 0.285^{a}	1.954 ± 0.143^{b}	0.209	<0.001
Root phosphorus (%)	1.273 ± 0.372^{f}	2.325 ± 0.164^{d}	1.88 ± 0.192^{e}	2.77 ± 193^{c}	3.746 ± 0.278^{a}	3.61 ± 0.204^{ab}	0.265	<0.001
Shoot phosphorus (%)	0.907 ± 0.13^{f}	1.948 ± 0.158^{d}	1.445 ± 0.287^{e}	2.538 ± 0.097^{c}	3.292 ± 0.197^{a}	3 ± 0.198^{b}	0.203	<0.001
Acidic phosphatase ($IU{\cdot}g^{-1}{\cdot}FW$)	0.236 ± 0.11^{d}	0.447 ± 0.12^{b}	0.318 ± 0.064^{c}	0.55 ± 0.123^{b}	0.708 ± 0.159^{a}	0.69 ± 0.111^{a}	0.128	<0.001
Alkaline phosphatase ($IU{\cdot}g^{-1}{\cdot}FW$)	0.332 ± 0.126^{d}	0.606 ± 0.144^{c}	0.587 ± 0.162^{c}	0.696 ± 0.219^{bc}	0.961 ± 0.123^{a}	0.821 ± 0.16^{ab}	0.173	<0.001

*Means within rows separated by different letters are significantly different based on the least significant difference test (LSD).

metabolites and various pharmacologically active compounds which is why the amount of anthocyanin had increased considerably in treated plants of saffron and *Gazania elegans*, respectively, probably the reason for increases in anthocyanin content. Lingua et al. [48] also confirmed that on inoculating AMF and *Pseudomonas* sp., useful secondary metabolites like anthocyanin along with phosphatase activity got increased.

Somewhat surprisingly, there were positive correlations among all of the traits studied, confirming that bioinoculants have an overall positive effect on all important traits for *Z. elegans*. Findings of Rouphael et al. [49] are in accordance with our results that biostimulants produce hydrolases and phosphatases enzymes more efficiently as compared to noninoculated treatments, and this could be the reason of improved phosphatase activity. Furthermore, an increased

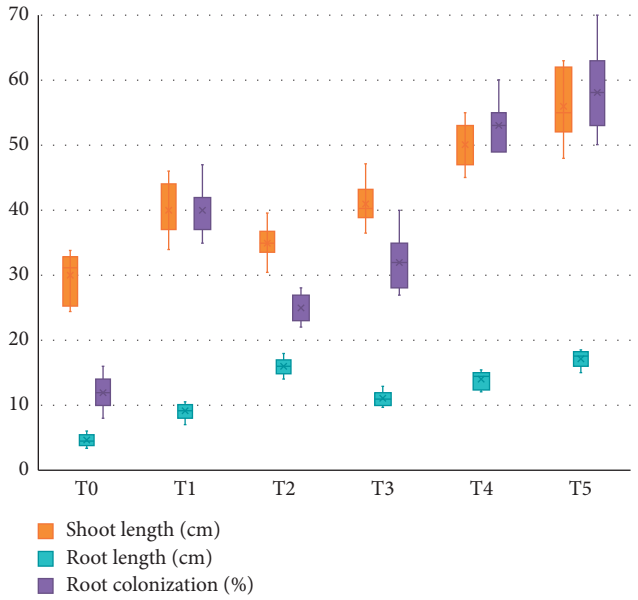

Figure 1: Shoot length, root length, and root colonization (%) across different treatments.

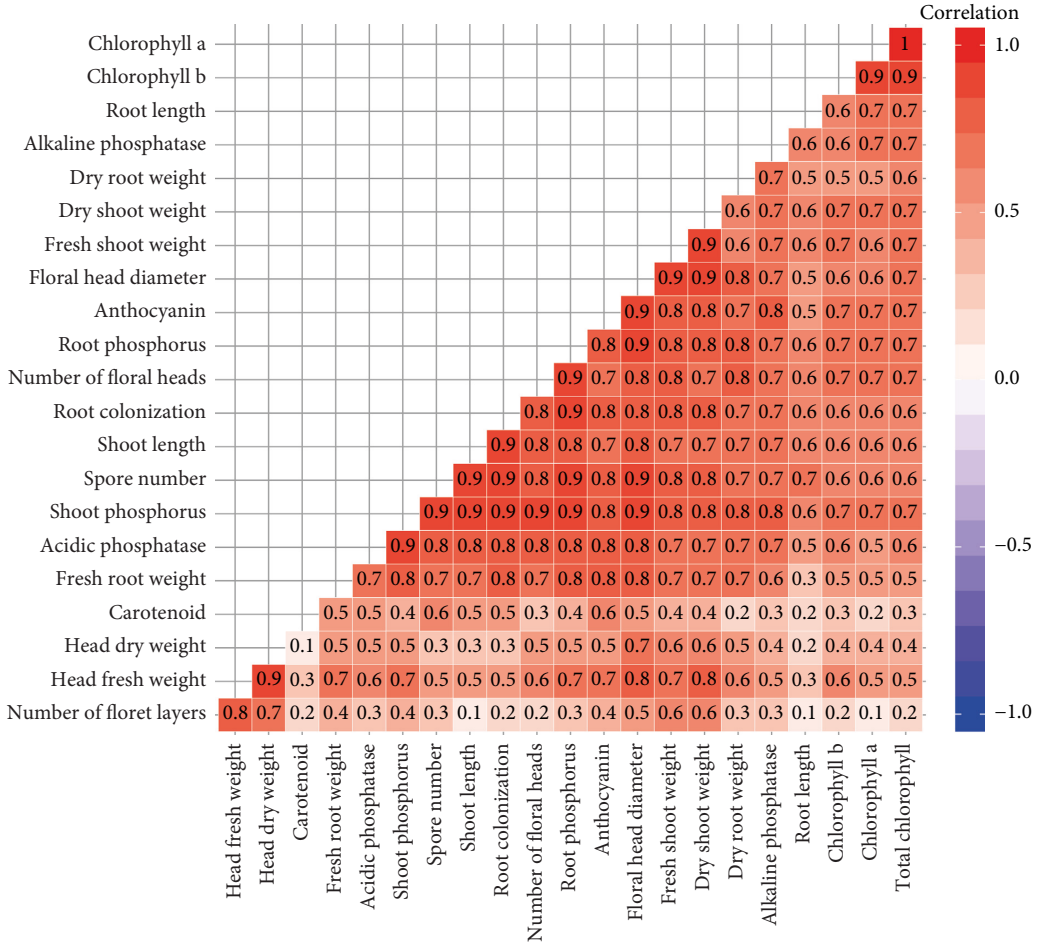

Figure 2: Pearson's correlation coefficients ($p < 0.05$) among the traits studied for *Z. elegans*.

phosphatase activity results in the rise of phosphorous content in the root and shoot even under nutrient deficit condition [42]. Fonte et al. [50] also concluded that biostimulants trigger phosphatase enzyme which solubilizes and mobilizes inorganic phosphorous in root microbiota. Inorganic phosphorous has a slow rate of solubilization; hence, its absorption

is slow but the application of AMF during floricultural practices increased the mobilization of phosphorous allowing co-transport of phosphorous toward host root [51]. It is already documented that AMF improves the phosphorous uptake even under phosphorous stress conditions [52].

AMF like *Glomus* and *Acaulospora* is the most dominant foremost mycorrhizal fungi almost ubiquitous in nature are now being used in the agriculture system around the world as phosphorus biostimulants [53]. So it is advisable that AM fungi can be seen as an exciting alternative to minimize crop dependency on phosphorous fertilizers, and their actual impact is currently strongly debated by several groups since contrasting effects of AM colonization on plant phosphorous nutrition have been reported [54–56].

5. Conclusions

In our experiment, inoculation treatment with biofertilizers of field-grown *Zinnia elegans* and conglomerate treatment of AMF and *Pseudomonas fluorescence* gave the best results in terms of plant growth and flower yield. As a result of the amalgamation of these biofertilizers, all the biochemical parameters do get increased. So it can be concluded that mycorrhizal fungi can increase the flower development by stimulating phytohormones and nutrient availability. Therefore, it is advised that the application of AMF during cultivation of floral products should be considered for the betterment of plant as well as soil ecosystem.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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