agriculture

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Inoculation with Mycorrhizal Fungi and Other Microbes to Improve the Morpho-Physiological and Floral Traits of *Gazania rigens* (L.) Gaertn

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Received: 14 January 2019; Accepted: 5 March 2019; Published: 8 March 2019

Abstract: In the present analysis, we evaluated the effect of different microbial inoculants on growth and floral responses of *Gazania rigens* (L.) Gaertn. Two prevailing arbuscular mycorrhizal fungi (AMF) i.e., *Funelliformis mosseae* and *Acaulospora laevis*, along with *Trichoderma viride* and *Pseudomonas fluorescens*, were examined in a pot experiment. Independent roles of these four microbes and their different combinations were used in sixteen treatments of *G. rigens*. The experiment was conducted in a polyhouse with five replicates per treatment in a randomized complete block design. The results of microbial inoculants were very effective for growth yield and flowering response over the control. Early flowering was noted in the combination of *F. mosseae*, *A. laevis* and *P. fluorescens*, which also showed the best results for flower head size, flower fresh and dry weight, total chlorophyll, carotene and phosphorus content. Arbuscular mycorrhiza (AM) root colonization (%) and AM spore number were also the highest for the same treatment, whereas the number of leaves, number of buds and root length were maximum in the combined treatment (*F. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens*). Overall, this study proposes that growers should consider microbial inoculants for the better yield and flower quality of *G. rigens*.

Keywords: arbuscular mycorrhizal fungi (AMF); *Pseudomonas fluorescens*; *Trichoderma viride*; microbes; bioinoculants; flower yield

1. Introduction

Gazania rigens (L.) Gaertn. (Family: Asteraceae) is an important annual flowering plant, commonly used as an ornamental garden plant [1]. Gazania has a long flowering duration from late spring to early summer [2]. The genus *Gazania* was named by Gaertner, honouring Theodorus Gaza, a 15th-century Italian scholar. *Gazania* along with other flowers, namely, *Geranium*, *Pelargonium*, *Watsonia* and *Clivia* were taken to Europe for cultivation from South Africa and from Europe, were subsequently used and popularized for the global horticultural trade [3]. *G. rigens* is one of the most common flowers on the market today, not only for ornamental purposes but also for its economic and medicinal importance [4,5] especially for antimicrobial activity of bioactive compounds present in the plant tissues [6]. The global floriculture industry is growing at a rapid pace, and recently, a great deal of significance has been given to the floriculture industry in India because of its foreign exchange earning potential, guaranteeing a higher rate of profits for rural individuals, and encouraging global trade [7].

A high demand for any ornamental plant is a major concern, especially under harsh urban conditions, which demand plants with high tolerance to various environmental stresses [8]. According to NHB (National Horticulture Board, India), the area under floral cultivation increased to about

248.51 thousand hectares in 2016–2017 while production of flowers is estimated around 1658 thousand tons of loose flowers and 472 thousand tons of cut flowers [9]. Therefore, to meet this high requirement, there should be some eco-friendly, low cost and sustainable strategy for intensive production and improvement of *G. rigens* cultivation. One of the methods is the use of bioinoculants, which are very beneficial, promising and favourable for soil [10–12].

Biofertilizers like beneficial fungi and certain phosphate-solubilizing bacteria (PSB), e.g., *Pseudomonas fluorescens*, are important for flower crop improvement [13]. Moreover, soil microbes from nutrient-poor ecosystems are known to form an important symbiosis with widely distributed plants to acquire essential macro and micronutrients [14]. Mycorrhizal association occurs between fungi and the roots of higher plants, and it has been acknowledged that arbuscular mycorrhizal fungi (AMF) provide phosphorus and other nutrients to plants [15]. The AMF approach ameliorates plant growth and can be an alternative to chemical fertilizers. Similarly, phosphorus-solubilizing bacteria have the ability to solubilize organic as well as inorganic phosphorus compounds by releasing organic acids or phosphatase enzymes [16,17]. Many studies have shown that the application of PSB has a synergistic effect when combined with AMF, as mycorrhizal exudates directly influence bacterial communities, enhancing water absorption of host plants [18–20].

Pseudomonas fluorescens, an aerobic multi-flagellated rod-shaped gram-negative plant growth-promoting rhizobacterium, plays a major role in plant growth development and in biological control of a wide range of pathogens [21]. It has been shown that *P. fluorescens* protects plant roots from the parasitic attack of *Fusarium* sp. and *Pythium* sp. [22–24]. Furthermore, *P. fluorescens* also produces many secondary metabolites that can act as a biocontrol agent for many fungal pathogens [25]. Similarly, plant roots secrete several ions, enzymes, free oxygen mucilage plus a diverse array of carbon-containing primary and secondary metabolites (organic acids, lipids, amino acids, flavonoids, sugars, aliphatic and aromatic compounds) that provide signals for root colonization [26]. *Trichoderma viride*, a non-mycorrhizal opportunistic, avirulent, free-living symbiont with phytostimulating activity, is widely used in floriculture practices for better plant growth [27,28]. *T. viride* enables root colonization, thereby enhancing plant root growth and metabolism by stimulating nutrient uptake. This can solve the problem of soil salinity, nutrition and drought [29]. Overall, these rhizospheric microbes bring considerable changes to plant metabolism and proteomes. These strategies are now being used at the nursery level for producing a better plant ideotype [30–32].

Biofertilizers like AMF, *Pseudomonas*, and *Trichoderma* can be an alternative source for enriching soil fertility and improving crop production [33]. It has been experimentally proven that AMF improves P and N uptake and other nutrients in plants [34]. Moreover, these microbes increase the mineralization and desorption of P from sparsely distributed sources and improve the internal P acquisition efficiency by lowering the P demand of plant growth [35]. Furthermore, these below-ground microbes govern the above-ground biodiversity, modifying the unfavourable environment to stimulate soil nutrient cycling [36]. AMF, being the most important biofertilizer, found in almost 90% of vascularophytes, are ubiquitous obligate symbionts in which fungal partners help the host by increasing the absorption of water and nutrients, and on the other hand, host plants act as a carbon source for the fungi [37].

Recently, it was concluded that combined effects of bioinoculants increase soil production, which ultimately lead to better growth of plants [38]. Earlier work on other ornamental species, namely, *Zinnia elegans*, *Tagetes patula and Salvia splendens*, showed progressive effects of AMF on growth and overall yield [17,39]. Therefore, we planned our study to scrutinize the effectiveness of AMF alone and in combination with *T. viride* and *P. fluorescens*, as no work has been done on *G. rigens* using these bioinoculants as biofertilizers in pot culture cultivation.

2. Materials and Methods

2.1. Soil Preparation

A pot experiment was conducted in a polyhouse of the Department of Botany, Kurukshetra University Kurukshetra, Haryana, India at 23 ± 5 °C and 45–65% relative humidity. A mixture of soil

and sand (3:1) was used for the experiment, with 64.2% sand, 21.8% silt, 3.9% clay, 0.042% N, 0.017% available P, 0.06% organic carbon and pH 7.4 ± 0 and was sieved through a 2-mm sieve and autoclaved (twice) at 121 °C to eradicate microorganisms.

2.2. Experimental Setup

2.2.1. AMF Inoculum Preparedness and Multiplication

Spores of the selected AMF (*Funelliformis mosseae* and *Acaulospora laevis* Gerd. & Trappe) were isolated from the rhizosphere of *G. rigens* plants growing in the botanical garden of Kurukshetra University, Kurukshetra, and identified using a key defined elsewhere [40]. Firstly, starter inoculum was prepared for both types of spores using a funnel technique [17], and then this starter inoculum was multiplied in standard pot culture using barley (*Hordeum vulgare*) as a host for 90 days because barley has a fibrous root system and short life span with the matching crop season to mass multiply the AMF inoculum.

2.2.2. Inoculum Preparation of *Trichoderma viride* Pers.

Inoculum preparation of *T. viride* was done by the soil dilution plate method [41] on potato dextrose agar medium and was identified based on a manual [42]. The cultures plates were incubated at 30 °C for 4 days. Then, the inoculum was multiplied using wheat-bran, saw-dust and water in a ratio of 3:1:1.

2.2.3. Inoculum Preparation of *Pseudomonas fluorescens* (MTCC No. 103)

Inoculum preparation of *P. fluorescens* was performed on nutrient broth medium prepared in sterilized water (incubated at 32 °C for 48 h). A culture of *P. fluorescens* was procured from the Institute of Microbial Technology (IMTECH, Chandigarh, India) and a concentration of 1×10^9 colony mL^{-1} was obtained and used for the inoculation.

2.3. Experimental Design

The experiment was laid out in a complete randomized block design with five replicates of each treatment including a control. Earthenware pots of 2 kg capacity were filled with sterilized soil: sand. AMF treatment containing 10% (w/w of soil) of the selected AMF inoculum having 645–685 AMF spores was added along with chopped AMF colonized root pieces of barley having an infection level of 80–85 percent. For *T. viride* infection, inoculum with a density of 3.4×10^8 CFU g^{-1} was added; CFU/g (colony-forming units per gram) for solids were determined using the formula CFU/g = (no. of colonies × dilution factor)/weight of the culture plate. *P. fluorescens* treatment involved the dipping of roots of 10-day-old plantlets of *G. rigens* for 5–10 min. Two plantlets were transplanted into each pot and were regularly watered. Hoagland's solution (without KH_2PO_4) was also provided at 14-day intervals. The list of a total of sixteen different treatment combinations is represented in Table 1.

Table 1. The following sixteen treatments (Tt) were maintained for the growth experiment.

Treatment	Constituents
Tt_1	Control (without any bio-inoculants)
Tt_2	*Funelliformis mosseae* (F)
Tt_3	*Acaulospora laevis* (A)
Tt_4	*Trichoderma viride* (T)
Tt_5	*Pseudomonas fluorescens* (P)
Tt_6	*F. mosseae* + *A. laevis* (F + A)
Tt_7	*F. mosseae* + *T. viride* (F + T)
Tt_8	*F. mosseae* + *P. fluorescens* (F + P)
Tt_9	*A. laevis* + *T. viride* (A + T)
Tt_{10}	*A. laevis* + *P. fluorescens* (A + P)

Table 1. *Cont.*

Treatment	Constituents
Tt_{11}	*T. viride* + *P. fluorescens* (T + P)
Tt_{12}	*F. mosseae* + *A. laevis* + *T. viride* (*F* + *A* + *T*)
Tt_{13}	*F. mosseae* + *A. laevis* + *P. fluorescens* (F + A + P)
Tt_{14}	*F. mosseae* + *T. viride* + *P. fluorescens* (F + T + P)
Tt_{15}	*A. laevis* + *T. viride* + *P. fluorescens* (A + T + P)
Tt_{16}	*F. mosseae* + *A. laevis* + *T. viride* + *P. fluorescens* (F + A + T + P), *consortium*

2.4. Characterization and Statistical Analysis

Plants were harvested after 90 days, and the length of the root, peduncle, and diameter of the floral head were measured. The fresh and dry weights of the shoot, root and floral head were weighed separately. Leaf area was measured by using a leaf area meter (Systronics 211, Ahmedabad, India). Numbers of leaves and floral heads were counted per plant per treatment. During the course of the experiment, the life-span of each floral head was recorded per plant per treatment. Chlorophyll and carotenoid contents were determined by Arnon's method [43]; briefly, 0.1 g of fresh leaf samples was crushed with 80% acetone using a mortar and pestle. The homogenate obtained was then centrifuged at 2000 rpm for about 15–20 min. The supernatant was collected, and absorbance was determined using a UV-Vis. spectrophotometer (Specord- 205 Analytik Jena AG, Jena, Germany) at 645 nm and 663 nm for chlorophyll a and chlorophyll b, respectively, and at 520 nm for carotenoids using 80% acetone as a blank.

The anthocyanin content in the florets was measured using Tsushida and Suzuki's method [44]; briefly, methanol with 1% hydrochloric acid was used. Thereafter, the absorbance-based anthocyanin concentration was determined at 530 nm using a UV-Vis. spectrophotometer (Specord- 205 Analytik Jena AG, Jena, Germany).

Acid and alkaline phosphatase activities were determined by Tabatabai and Bremner's method [45]; briefly, 1 g of washed roots was homogenized in ice-cold sodium acetate buffer (0.1 M with pH 4.8) for acidic phosphatase activity, and sodium bicarbonate buffer (0.05 N with pH 10) was used for the estimation of the alkaline phosphatase activity. After centrifugation at 10,000 rpm for 15 min, the supernatant was used for assaying phosphatase activity. Root and shoot phosphorus contents in the samples were analyzed by the vanadomolybdo phosphoric yellow colour method [46]. AMF spores were extracted from the rhizospheric soil of gazania plants using wet sieving and a decanting technique [47] and then counted by the gridline intersect method [48]. AMF root colonization was done using 0.01% trypan blue stain after cleaning the root pieces with 10% KOH [49], and the quantification of colonized roots was completed using the root slide technique [50]. AM Root Colonization (%) was determined based on the formula 100 × (number of root segments colonized/total number of root segments).

Means of each treatment were subjected to analysis of variance (ANOVA) to determine differences among the sixteen treatments. The significance of differences among treatment means was evaluated using Duncan's multiple range test (DMRT) for comparison of variance separated with (least significant difference) (LSD) as a post hoc test through SPSS software (11.5 version) [51].

3. Results

Highly significant differences ($p < 0.001$) were present in the mean values of the groups that constituted the sixteen different treatments applied to the gazania plants (Table 2). The application of bioinoculants showed a significant and positive effect on the morpho-physiological as well as phytochemical traits of the *Gazania rigens* (L.) Gaertn (Table 2). The effect of bioinoculants on several morphological parameters of *G. rigens* was statistically significant (Table 2. Both shoot and root weight were maximum in Tt_{13}, F + A + P as depicted in Table 2. The longest root length was noted in Tt_{16}, F + A + T + P i.e., combined treatment (7.27 ± 0.2) followed by Tt_{13} (7.12 ± 0.19). The highest number of leaves were found in Tt_{16} (61 ± 2.23) followed by Tt_{13} (52 ± 5.24), while for leaf area, the Tt_{13} treatment (21.2 ± 0.24) showed the best result followed by Tt_6, F + A (20.74 ± 0.43) (Table 2).

From Table 2, it is clear that AMF root colonization and AMF spore number followed the same trend, where Tt_{13} (spore number, 127 ± 8.7; root colonization 75 ± 4.6) proved to be the best treatment followed by Tt_{16} (spore number, 121 ± 8.9; root colonization 68 ± 8.8) (Table 2). Plants inoculated with bioinoculants showed significant flowering as compared to un-inoculated controls (Table 3). Plant inoculated with F + A + P bloomed first whereas un-inoculated plants took 17 days longer after all the inoculated plants flowered. The inoculated plants not only flowered first but also had a significantly higher number of flowers. Tt_{16} (F + A + T + P), i.e., consortium, showed the highest number of flowers (11 ± 1.58) (Table 3). The peduncle length among inoculated plants was longest in Tt_{13} (F + A + P) (Table 3). The diameter and weight of the floral head also showed the same trend, being the heaviest in Tt_{13} followed by the consortium (Table 3).

The differences among the AM root colonization (%) are displayed in Figure 1. Four treatments comprising the control, *Pseudomonas fluorescence*, *Trichoderma viride* and both together did not have any AM root colonization, whereas, the highest value, i.e., above 80% of the root, was observed in the FAP treatment (Figure 1).

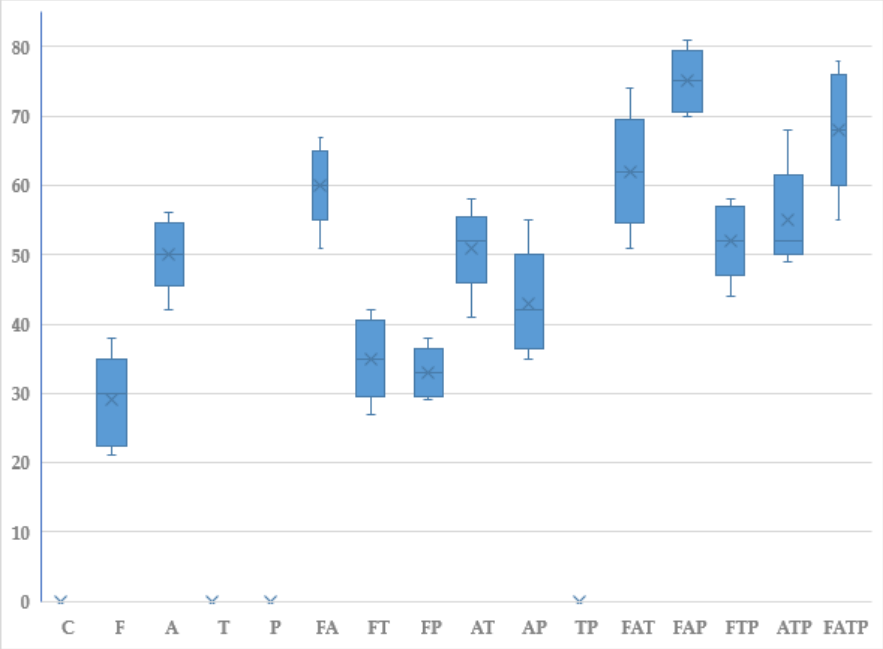

Figure 1. AM root colonization (%) in the sixteen treatments. C—Control, F—*Funelliformis mosseae*, A—*Acaulospora laevis*, T—*Trichoderma viride*, and P—*Pseudomonas fluorescence*.

Further, in the case of total chlorophyll, carotenoid and anthocyanin contents, as depicted in Table 4, Tt_{13} (F + A + P) showed the best result as compared to other treatments, with the lowest values in the control. Total chlorophyll (chl) and carotenoid contents were maximum in the F + A + P treatment (chlorophyll-a, 1.108 ± 0.168; chlorophyll-b, 0.777 ± 0.118; carotenoid, 0.123 ± 0.027) (Table 4). The floral anthocyanin content was highest under the combined effect of F + A + P, Tt_{13} (35.47 ± 0.815) as shown in Table 4, followed by the consortium treatment, Tt_{16} (34.95 ± 0.851). Similarly, all the biochemical parameters illustrated in Table 3 showed a better result in the treated plants as compared to the control. The total phosphorus content was highest in the Tt_{13} (F + A + P) (shoot phosphorus, 1.313 ± 0.078; root phosphorus, 1.56 ± 0.125) followed by the consortium treatment, Tt_{16} (shoot phosphorus, 1.215 ± 0.256; root phosphorus, 1.397 ± 0.316) (Table 4). Compared to enhanced phosphatase activity, identical results were shown in the Tt_{13} treatment (acidic phosphatase, 1.565 ± 0.196; alkaline phosphatase, 2.531 ± 0.204) followed by the consortium treatment (acidic phosphatase, 1.502 ± 0.209; alkaline phosphatase, 2.314 ± 0.114) as shown in Table 4.

Table 2. Effect of bioinoculants on morphological parameters of *Gazania rigens*.

Treatments	Fresh Shoot Weight (g)	Dry Shoot Weight (g)	Fresh Root Weight (g)	Dry Root Weight (g)	Root Length (cm)	Number of Leaves	Leaf Area (cm^2)	AM Spore Number	AM Root Colonization (%)
Control	$6.01 \pm 1.09^{k‡}$	1.069 ± 0.12^{k}	1.8 ± 0.34^{j}	0.3 ± 0.05^{k}	1.97 ± 0.36^{i}	9 ± 2.23^{l}	8.3 ± 0.61^{k}	0 ± 0^{g}	0 ± 0^{g}
Funelliformis mosseae (F) †	20.3 ± 1.88^{hi}	15.26 ± 0.83^{h}	2.76 ± 0.19^{g}	0.89 ± 0.05^{h}	4.9 ± 0.26^{e}	18 ± 3.08^{j}	14.5 ± 0.42^{f}	50 ± 8.2^{f}	29 ± 6.7^{f}
Acaulospora laevis (A)	21.12 ± 2.04^{h}	15.921 ± 1.31^{h}	2.54 ± 0.15^{gh}	0.65 ± 0.08^{i}	5.8 ± 0.22^{cd}	29 ± 3.16^{hi}	14.8 ± 0.36^{f}	57 ± 4.6^{f}	50 ± 5.2^{de}
Trichoderma viride (T)	15.51 ± 2.45^{j}	11.63 ± 0.85^{j}	2.2 ± 0.18^{i}	0.42 ± 0.02^{j}	3.1 ± 0.35^{h}	13 ± 1.22^{k}	12.5 ± 0.34^{j}	0 ± 0^{g}	0 ± 0^{g}
Pseudomonas fluorescence (P)	18.53 ± 1.71^{i}	13.36 ± 0.55^{i}	2.3 ± 0.17^{h}	0.48 ± 0.02^{ij}	3.4 ± 0.25^{gh}	15 ± 1.58^{jk}	13.7 ± 0.21^{h}	0 ± 0^{g}	0 ± 0^{g}
FA	36.65 ± 1.35^{b}	32.17 ± 0.89^{a}	2.8 ± 0.16^{g}	1.03 ± 0.11^{gh}	7 ± 0.36^{ab}	30 ± 3.39^{hi}	20.74 ± 0.43^{a}	91 ± 8.4^{c}	60 ± 5.9^{c}
FT	32.73 ± 1.3^{c}	26.85 ± 0.59^{c}	3.5 ± 0.26^{f}	2.05 ± 0.36^{e}	4.11 ± 0.36^{f}	32 ± 3.16^{gh}	16.28 ± 0.19^{d}	76 ± 6.6^{de}	35 ± 5.8^{f}
FP	31.11 ± 1.63^{cd}	23.69 ± 0.66^{d}	4.83 ± 0.16^{c}	2.98 ± 0.27^{c}	3.7 ± 0.38^{fg}	33 ± 3.8^{fgh}	18.3 ± 0.35^{b}	70 ± 7.3^{j}	33 ± 3.6^{f}
AT	30.08 ± 1.47^{de}	22.55 ± 0.62^{e}	4.39 ± 0.29^{d}	2.54 ± 0.12^{d}	5.6 ± 0.45^{de}	35 ± 4.12^{efg}	13.9 ± 0.46^{h}	85 ± 8^{cd}	51 ± 6.2^{d}
AP	35.27 ± 2.6^{b}	30.06 ± 0.86^{b}	5.83 ± 0.16^{b}	3.86 ± 0.16^{b}	4.8 ± 0.48^{e}	38 ± 2.91^{de}	15.5 ± 0.26^{e}	78 ± 8.9^{de}	43 ± 7.7^{e}
TP	22.72 ± 2.39^{h}	17.06 ± 0.59^{g}	4.01 ± 0.25^{de}	2.15 ± 0.14^{e}	3.5 ± 0.1^{gh}	27 ± 4.3^{i}	14 ± 0.13^{g}	0 ± 0^{g}	0 ± 0^{g}
FAT	25.7 ± 2.13^{g}	20.55 ± 0.44^{f}	2.9 ± 0.08^{g}	1.05 ± 0.13^{gh}	6.67 ± 0.23^{b}	41 ± 4^{cd}	13 ± 0.36^{i}	113 ± 11.5^{b}	62 ± 8.5^{bc}
FAP	39.84 ± 2.75^{a}	32.64 ± 0.74^{a}	6.5 ± 0.15^{a}	4.5 ± 0.24^{a}	7.12 ± 0.19^{a}	52 ± 5.24^{b}	21.2 ± 0.24^{a}	127 ± 8.7^{a}	75 ± 4.6^{a}
FTP	27.42 ± 1.62^{fg}	21.21 ± 0.94^{f}	3.93 ± 0.23^{e}	1.25 ± 0.08^{g}	6.1 ± 0.5^{c}	37 ± 3.67^{def}	16.6 ± 0.39^{d}	89 ± 10.5^{c}	52 ± 5.4^{d}
ATP	27.98 ± 1.39^{efg}	22.71 ± 0.67^{e}	3.63 ± 0.11^{ef}	1.76 ± 0.11^{f}	5.2 ± 0.35^{g}	45 ± 1.58^{c}	14.5 ± 0.29^{f}	95 ± 10.6^{c}	55 ± 7.5^{cd}
FATP	28.82 ± 1.89^{def}	22.58 ± 0.43^{e}	5.82 ± 0.13^{b}	3.8 ± 0.25^{b}	7.27 ± 0.2^{a}	61 ± 2.23^{a}	17.17 ± 0.48^{c}	121 ± 8.9^{ab}	68 ± 8.8^{ab}
F-ratio	101.639	605.75	104.491	315.15	112.983	91.697	359.306	104.293	170.635
Probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

† F—*Funelliformis mosseae*, A—*Acaulospora laevis*, T—*Trichoderma viride*, P—*Pseudomonas fluorescence*; ± —Standard deviation; FW—Fresh Weight, ‡ values in columns followed by the same letter are not significantly different, $p \leq 0.05$, LSD.

Table 3. Effect of bioinoculants on floral parameters of *Gazania rigens*.

Treatments	No. of Floral Heads Per Plant	Peduncle Length (cm)	Diameter of Flower Head (cm)	Flower Fresh Weight (g)	Flower Dry Weight (g)	Flower Age on the Plant (Days)
Control	$2 \pm 0.707^{h‡}$	1.88 ± 0.609^{i}	2.7 ± 0.46^{k}	2.46 ± 0.079^{j}	0.73 ± 0.091^{j}	2 ± 0.7^{h}
Funelliformis mosseae (F) †	5 ± 1.581^{ef}	4.5 ± 0.412^{de}	7.51 ± 0.71^{def}	3.4 ± 0.064^{def}	1.68 ± 0.088^{ef}	5 ± 1.41^{efg}
Acaulospora laevis (A)	6 ± 0.707^{de}	4.2 ± 0.291^{fg}	6.5 ± 0.68^{ghi}	3.26 ± 0.113^{gh}	1.53 ± 0.041^{gh}	6 ± 1^{cde}
Trichoderma viride (T)	3 ± 0.707^{gh}	3.04 ± 0.304^{h}	5.2 ± 0.58^{j}	3.1 ± 0.112^{hi}	1.38 ± 0.031^{i}	3 ± 1.22^{gh}
Pseudomonas fluorescence (P)	4 ± 1.581^{fg}	3.62 ± 0.319^{g}	5.7 ± 0.83^{ij}	3.62 ± 0.055^{c}	1.45 ± 0.015^{hi}	4 ± 2^{fgh}
FA	8 ± 0.707^{a}	6.1 ± 0.337^{b}	8 ± 0.79^{cde}	3.7 ± 0.067^{c}	1.9 ± 0.064^{d}	7 ± 1.58^{bcd}
FT	6 ± 0.707^{de}	5.2 ± 0.447^{c}	8.5 ± 0.71^{bcd}	3.34 ± 0.14^{efg}	1.98 ± 0.066^{d}	6 ± 1.58^{cde}
FP	7 ± 1.224^{cd}	4.8 ± 0.524^{cde}	7 ± 0.61^{fgh}	3.5 ± 0.113^{d}	1.61 ± 0.08^{fg}	6 ± 1^{cde}
AT	7 ± 0.707^{cd}	4.3 ± 0.339^{ef}	8.2 ± 0.36^{cde}	3.3 ± 0.14^{fg}	1.77 ± 0.05^{e}	7 ± 1.73^{bcd}
AP	8 ± 1^{bc}	6.2 ± 0.587^{b}	6.1 ± 0.22^{hij}	3.27 ± 0.046^{gh}	1.57 ± 0.09^{g}	7 ± 1.22^{bcd}
TP	5 ± 0.707^{ef}	6 ± 0.474^{b}	6 ± 0.5^{ij}	4.07 ± 0.04^{b}	1.55 ± 0.146^{g}	5 ± 0.7^{efg}
FAT	7 ± 1.224^{cd}	4.5 ± 0.463^{de}	8.9 ± 0.35^{abc}	4.32 ± 0.041^{a}	2.2 ± 0.015^{c}	9 ± 1^{ab}
FAP	8 ± 1.224^{bc}	7.3 ± 0.412^{a}	9.6 ± 1.09^{a}	4.43 ± 0.015^{a}	2.56 ± 0.015^{a}	10 ± 1.58^{a}
FTP	8 ± 0.707^{bc}	4.7 ± 0.158^{cde}	8.1 ± 0.54^{cde}	3.43 ± 0.022^{de}	1.71 ± 0.027^{e}	8 ± 1.58^{abc}
ATP	9 ± 1.224^{b}	5.01 ± 0.312^{cd}	7.3 ± 0.73^{efg}	3.41 ± 0.051^{def}	1.69 ± 0.015^{ef}	8 ± 2^{abc}
FATP	11 ± 1.58^{a}	5.8 ± 0.33^{b}	9.3 ± 1.43^{ab}	4.16 ± 0.027^{b}	2.43 ± 0.043^{b}	10 ± 2.54^{a}
F-ratio	20.813	46.529	29.819	152.091	194.08	11.68
Probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

† F—*Funelliformis mosseae*, A—*Acaulospora laevis*, T—*Trichoderma viride*, P—*Pseudomonas fluorescence*; ±—Standard deviation; FW—Fresh Weight, ‡ values in a column followed by the same letter are not significantly different, $p \leq 0.05$, LSD.

Table 4. Effect of bioinoculants on biochemical aspects of *Gazania rigens*.

Treatments	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total Chlorophyll (mg/g FW)	Total Carotenoids (mg/g FW)	Shoot Phosphorus Content (%)	Root Phosphorus Content (%)	Acidic Phosphatase (IU g^{-1} FW)	Alkaline Phosphatase (IU g^{-1} FW)	Anthocyanin Content (mg/100 g FW)
Control	0.233 ± 0.08^{g}	0.121 ± 0.036^{g}	0.354 ± 0.075^{l}	0.012 ± 0.004^{k}	0.321 ± 0.105^{i}	0.395 ± 0.088^{h}	0.6 ± 0.019^{h}	1.411 ± 0.14^{i}	17.4 ± 1.398^{i}
Funelliformis mosseae (F) †	0.59 ± 0.11^{def}	0.402 ± 0.112^{ef}	0.992 ± 0.152^{ij}	0.037 ± 0.014^{ijk}	0.777 ± 0.18^{efgh}	0.845 ± 0.119^{efg}	0.925 ± 0.121^{efg}	1.777 ± 0.109^{gh}	26.3 ± 0.988^{e}
Acaulospora laevis (A)	0.615 ± 0.153^{de}	0.417 ± 0.11^{ef}	1.032 ± 0.259^{hi}	0.049 ± 0.011^{ghi}	0.7 ± 0.235^{fgh}	0.843 ± 0.139^{efg}	1.003 ± 0.134^{defg}	1.875 ± 0.088^{efg}	28.3 ± 0.948^{d}
Trichoderma viride (T)	0.4 ± 0.068^{efg}	0.201 ± 0.038^{fg}	0.601 ± 0.087^{k}	0.02 ± 0.009^{jk}	0.538 ± 0.123^{gh}	0.611 ± 0.125^{gh}	0.789 ± 0.12^{gh}	1.656 ± 0.083^{h}	20.8 ± 0.923^{h}
Pseudomonas fluorescence (P)	0.387 ± 0.087^{fg}	0.32 ± 0.108^{fg}	0.707 ± 0.071^{jk}	0.025 ± 0.004^{ijk}	0.601 ± 0.139^{fgh}	0.695 ± 0.218^{fg}	0.802 ± 0.132^{fg}	1.687 ± 0.113^{gh}	21.11 ± 0.998^{f}
FA	0.702 ± 0.137^{cd}	0.489 ± 0.173^{def}	1.191 ± 0.212^{efgh}	0.087 ± 0.019^{cde}	0.829 ± 0.2^{cdef}	0.892 ± 0.13^{ef}	1.082 ± 0.171^{cde}	2.075 ± 0.134^{cd}	32.23 ± 0.619^{bc}
FT	0.666 ± 0.153^{cd}	0.432 ± 0.137^{ef}	1.098 ± 0.047^{ghi}	0.055 ± 0.014^{fgh}	0.813 ± 0.188^{defgh}	0.885 ± 0.167^{ef}	0.987 ± 0.184^{defg}	1.978 ± 0.091^{def}	27.1 ± 1.049^{e}
FP	0.767 ± 0.15^{bcd}	0.571 ± 0.197^{abcde}	1.338 ± 0.295^{defg}	0.062 ± 0.017^{fg}	0.906 ± 0.248^{cdef}	0.977 ± 0.172^{de}	1.013 ± 0.108^{de}	1.789 ± 0.118^{fgh}	29.1 ± 0.605^{d}
AT	0.887 ± 0.175^{abc}	0.6 ± 0.201^{abcde}	1.487 ± 0.332^{bcde}	0.076 ± 0.016^{def}	0.927 ± 0.221^{cdef}	1.008 ± 0.098^{cde}	1.025 ± 0.103^{de}	2.028 ± 0.129^{de}	31.79 ± 0.539^{c}
AP	0.875 ± 0.21^{abc}	0.543 ± 0.137^{bcde}	1.418 ± 0.325^{cdef}	0.071 ± 0.021^{efg}	0.957 ± 0.198^{bcdef}	1.039 ± 0.195^{cde}	1.051 ± 0.15^{de}	2.003 ± 0.148^{de}	32.09 ± 0.617^{bc}
TP	0.426 ± 0.104^{efg}	0.505 ± 0.154^{cdef}	0.931 ± 0.254^{ij}	0.031 ± 0.015^{hijk}	0.888 ± 0.175^{cdef}	0.951 ± 0.185^{def}	0.904 ± 0.124^{efg}	1.806 ± 0.143^{fgh}	24.2 ± 0.729^{f}
FAT	1.004 ± 0.273^{a}	0.701 ± 0.156^{abc}	1.706 ± 0.39^{abc}	0.09 ± 0.024^{bcde}	1.111 ± 0.295^{abc}	1.303 ± 0.336^{b}	1.321 ± 0.213^{bc}	2.243 ± 0.232^{bc}	33.03 ± 0.888^{b}
FAP	1.108 ± 0.168^{a}	0.777 ± 0.118^{a}	1.885 ± 0.086^{a}	0.123 ± 0.027^{a}	1.313 ± 0.078^{a}	1.56 ± 0.125^{a}	1.565 ± 0.196^{a}	2.531 ± 0.204^{a}	35.47 ± 0.815^{a}
FTP	0.951 ± 0.226^{ab}	0.654 ± 0.118^{abcd}	1.605 ± 0.25^{abcd}	0.1 ± 0.02^{abcd}	1.031 ± 0.234^{bcde}	1.2 ± 0.224^{bcd}	1.175 ± 0.126^{bcd}	2.087 ± 0.19^{cd}	31.24 ± 0.859^{c}
ATP	0.989 ± 0.206^{ab}	0.686 ± 0.166^{abcd}	1.675 ± 0.348^{abc}	0.105 ± 0.025^{abc}	1.073 ± 0.234^{abcd}	1.256 ± 0.203^{bc}	1.281 ± 0.163^{cd}	2.102 ± 0.19^{cd}	31.73 ± 0.751^{c}
FATP	1.006 ± 0.109^{a}	0.751 ± 0.116^{sb}	1.817 ± 0.127^{ab}	0.113 ± 0.015^{ab}	1.215 ± 0.256^{ab}	1.397 ± 0.316^{ab}	1.502 ± 0.209^{ab}	2.314 ± 0.114^{b}	34.95 ± 0.851^{a}
F-ratio	14.139	8.694	18.624	19.659	8.314	12.409	14.609	19.113	187.155
Probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

† F—*Funelliformis mosseae*, A—*Acaulospora laevis*, T—*Trichoderma viride*, P—*Pseudomonas fluorescence*; ±—Standard deviation; FW—Fresh Weight, ‡ values in columns followed by the same letter are not significantly different, $p \leq 0.05$, LSD.

In summary, the best three inoculation treatments among the total sixteen are presented in Table 5. Overall, the treatment with *Funelliformis mosseae* (F), *Acaulospora laevis* (A), and *Pseudomonas fluorescens* (P) was frequently the best treatment for the traits studied.

Table 5. Top three treatments for morphological, floral and biochemical traits studied in *Gazania rigens*.

Traits	Treatments *
Fresh shoot weight (g)	FAP FA FT
Dry shoot weight (g)	FAP FA FT
Fresh root weight (g)	FAP FATP GP
Dry root weight (g)	FAP FATP GP
Root length (cm)	FATP FAP FAT
Number of Leaves	FATP FAP ATP
Leaf area (cm^2)	FAP FA GP
AM spore number	FAP FATP FAT
AM root colonization (%)	FAP FATP FAT
No. of floral heads per plant	FATP ATP FAP
Peduncle length (cm)	FAP AP FA
Diameter of flower head (cm)	FAP FATP FAT
Flower fresh weight (g)	FAP FAT FATP
Flower dry weight (g)	FAP FATP FAT
Flower age on the plant (days)	FAP FATP FAT
Chlorophyll a (mg/g FW)	FAP FATP FAT
Chlorophyll b (mg/g FW)	FAP FATP FAT
Total chlorophyll (mg/g FW)	FAP FATP FAT

Table 5. *Cont.*

Traits	Treatments *
Total carotenoids (mg/g FW)	FAP FATP ATP
Shoot Phosphorus Content (%)	FAP FATP FAT
Root Phosphorus Content (%)	FAP FATP FAT
Acidic Phosphatase (IU g^{-1} FW)	FAP FATP FAT
Alkaline Phosphatase (IU g^{-1} FW)	FAP FATP FAT
Anthocyanin content (mg/100 g FW)	FAP FATP FAT

* *Funelliformis mosseae* (F), *Acaulospora laevis* (A), *Trichoderma viride* (T), and *Pseudomonas fluorescens* (P).

4. Discussion

Gazania is also known as the treasure flower and originates from South Africa. The flowers of gazania possess a long flower life; therefore, in gardens they are generally used for bedding, edging and as well as for mass planting [52]. The present study demonstrated that bioinoculant-treated plants grew well, and flowering varied with different combinations used. It is clear-cut from the data that bioinoculant-infected plants showed better results in growth and mineral nutrition compared to un-inoculated control plants. The hyphopodia of AMF enter the root cortex to obtain carbon from the host plant and in return assist the plant with more uptake of nutrients, especially P, which is necessary for the synthesis of nucleic acids [53], enhancing vegetative growth [54,55]; this might be the reason for the higher weight (fresh/dry root/shoot weight) of the plants. Along these lines, our study found that the combination of AMF (*F. mosseae* and *A. laevis*) with *Pseudomonas fluorescence* had the best result among the bioinoculants used. Similarly, it is also confirmed that bioinoculated plants of *Tagetus patula* showed increased leaf area and shoot and root fresh and dry weight [56].

The effectiveness of bioinoculants can differ with inoculant forms, soil properties and environmental conditions [57]. Polyhouse conditions were selected for the experiment and soil samples were autoclaved. Generally, *Funelliformis mosseae* and *Acaulospora laevis* have a different magnitude of root colonization because the extent of absorption of water and minerals might differ among treatments [58,59]. If the level of absorbed minerals is different, that could lead to a variation in plant growth parameters we considered in this study [60]. Similarly, *Trichoderma viride* and *Pseudomonas fluorescence* possess a different absorption rate [61]. Furthermore, the AMF produce some organo-polysaccharides exudates that are crucial for soil porosity and soil quality [62], whereas, if AMF amalgamates with *Trichoderma viride* and *Pseudomonas fluorescence*, the amount and form of these exudates become more commendable [63]. Hyphae of mycorrhizal fungi-infected roots can penetrate deep in the phosphorus depletion zone and make phosphorus easily accessible for plants [64].

PSB can solubilize orthophosphates and AMF roots can easily absorb these orthophosphates. Therefore, in our study, different combinations altered the absorption rate [65]. When the number of absorbed minerals differs, plants have diverse amounts of phytoaccumulation and phytohormones [66]. This change is directly linked to the photosynthetic rate in the plant combinations [67]. Usually, the amount of exudates is linked with the amount of carbon the AMF is receiving from its host [68]. Hence, different combinations of bioinoculants varied in their response, and plants showed an overall diverse characterization.

The AMF root colonization (%) and AMF spore number were significantly more developed in the treated plants as compared to the control. Furthermore, the treatments T, P and TP showed no AMF root colonization (%) because both of the microorganisms (T and P) belong to the non-infectious group and are mycorrhizal-activating microbes. *Trichoderma viride* is a mycorrhizal helper fungus; it assists in the colonization of already present AMF but never colonizes on its own. Similarly, Pseudomonas is phosphate-solubilizing bacteria [69]. Moreover, the soil used was autoclaved and the experiment was performed under polyhouse conditions, so there were limited chances of infection.

These results are in line with previous studies carried out on flowering plants [70–72]. Findings of Scagel [73] for yellow zephyr lily are correlated with our work as the inoculated plants showed earlier flowering as compared to the control. This might be due to the enhanced production of auxin and gibberellin that induces bud production due to higher levels of potassium absorption by the plant [74–76]. Similarly, an increased flower number was also recorded for the *Tagetes* sp., *Zinnia* sp. and *Callistephus* sp., with the use of AMF treatment [77–79]. In treatments with bioinoculants plant characters like diameter, fresh and dry weight of flowers were better than those of the un-inoculated plants and this might be due to higher absorption of water and minerals like P, Fe and Zn [80]. In this direction, Vaingankar and Rodrigues [81] showed a similar increase in weight of *Crossandra infundibuliformis* flowers when AMF treatment was applied. Our results showed the enhancement in total chlorophyll and carotenoid contents in the treated plants, probably due to increased stomatal conductance and photosynthesis [82]. Another reason could be an increase in the number and size of the chloroplasts [83].

In the case of shoot and root P concentration, a mycorrhizal effect was evident, because the AMF-associated roots produced some acid phosphatases and hydrolase enzymes that increased phosphate availability in the rhizosphere [84,85]. These results correspond with the work of Nowak [86], where *Pelargonium hortorum* grew under low NPK supply but with mycorrhizal fungi, an increase in the P content was noted. Acid and alkaline phosphatase activities increased in the treatment with AMF in combination with *P. fluorescence*. This augmentation may be attributed to increased activity of phosphatase enzyme, which results in mineralization of inorganic phosphorus from organic compounds [87]. Previously, a high level of phosphatase activity was reported by using histo/cytochemical staining techniques, proving that AMF increase alkaline phosphatase activity [88–90]. Moreover, it has been suggested that biofertilizers possess great potential for the horticulture industry [91]. Likewise, this study proposes that growers should consider microbial inoculants for the better yield and flower quality of *G. rigens*.

5. Conclusions

Arbuscular mycorrhizal fungi (AMF) benefit host plants symbiotically in many aspects such as the uptake of phosphorus and other nutrients, increased plant growth hormone production, plant height, total leaf area, fresh/dry weight of shoots and roots, number and weight of flowers, the rate of colonization and arbuscular richness and also provide resistance to phytopathogens. Being a semi-arid region, Haryana faces a problem in the large production of the floriculture industry; therefore, the use of AMF may be implemented in nursery practices for improved crop growth and yield. Overall, we have concluded that bioinoculant treatment is promising for *G. rigens;* furthermore, bioinoculants are eco-friendly and inexpensive compared to chemical fertilizers. This study was carried out under controlled conditions; therefore, it is not known how plants will be affected under natural conditions where climatic factors and microbes have to interact more closely with each other to yield a plant ideotype.

Author Contributions: I.S. and A.A. conceived and designed the project. A.A. supervised the study. I.S. performed the experiments. I.S. and P.K. analyzed the data. I.S., A.A. and P.K. wrote the paper and corrected the final draft. All authors read and approved the final manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors are thankful to the anonymous reviewers for their careful reading of the manuscript and providing insightful suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

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