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Glomus mosseae and *Pseudomonas fluorescens* Application Sustains Yield and Promote Tolerance to Water Stress in *Helianthus annuus* L.

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Abstract: The inoculation of sunflower (*Helianthus annuus* L.) plants with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) can significantly enhance its growth and yield in a sustainable manner. Drought tolerance is mediated by a combination of direct AMF and PGPR benefits that boost the plant's natural ability to cope with stress, whereas drought mitigation is mediated by indirect AMF and PGPR benefits and increased water uptake. An experiment was carried out to demonstrate the interactive effects of AMF (*Glomus mosseae*) alone or in association with PGPR (*Pseudomonas fluorescens*) under water-stressed conditions in order to assess their biofertilizer efficiency. Accordingly, various morphological and biochemical parameters were studied, and the results suggested that all the co-inoculation treatments displayed beneficial effects. Still, the combination of *G. mosseae* + *P. fluorescens* showed the maximum increment in all the parameters considered, i.e., plant height and weight, leaves length and width, number of leaves per plant, specific leaf weight, relative leaf water content (RLWC), photosynthetic efficiency, seed length, width, and area, seed yield per plant, number of seeds per flower, days to 50% flowering, days to maturity, flower and head diameter, harvest index, oil content, fatty acid composition (palmitic acid, oleic acid, stearic acid, and linoleic acid), and total yield. The improvement in different parameters may be attributed to the increased availability of nutrients due to the symbiotic association of AMF and PGPR with plant roots along with enhanced root structures for more water absorption under stressed conditions. Therefore, the results suggested that they offer a promising bio-control strategy for crop protection as biofertilizers combined in one formulation.

Keywords: sunflower; mycorrhizal fungi; *Pseudomonas*; water stress; yield



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1. Introduction

Sunflower is one of the most valuable sources of edible oils known for its monounsaturated fats. The oil content of about 36–52% and protein content of 28–32% are found in sunflower seeds [1]. It also contains vitamins A, D, E, and K, which renders them beneficial in averting heart diseases [2,3]. Therefore, to enhance the productivity of this important oilseed crop, various biotic or abiotic factors that could affect its growth and development should be considered carefully. Drought (or water deficit) stress is one of these limiting abiotic factors that has received a lot of attention because it is the most likely constraint for agricultural productivity [4]. Drought happens when the plant's water requirements are not fully met, and this occurs when the amount of transpired water exceeds the amount of water taken up by the roots, which is caused by insufficient precipitation, low groundwater levels, or water retention by soil particles [5,6]. Water scarcity causes stomatal closure, which reduces CO₂ influx, lowering photosynthetic activity and carbon partitioning [7],

as well as plant productivity and agricultural produce. Further, increasing environmental problems triggered by chemical fertilizers have created the need to use biofertilizers for sustainable crop production [4]. In this context, plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) represent the two most important groups of beneficial rhizospheric micro-organisms which may act as biofertilizers [5]. The micro-organisms capable of colonizing the rhizosphere may be categorized based on their way of interaction with plants, and particularly with roots, some act as pathogens while others produce beneficial effects [6,7].

The beneficial effects of PGPR and AMF on sunflower plants have been well-documented through many studies. Mycorrhizal fungi, such as *Glomus mosseae*, help in increasing water and mineral uptake by roots, subsequently improving the soil structure [8,9]. The other significant benefit of AMF includes protecting the host plant from pathogenic infections caused by various soil microbes such as bacteria, fungi, and nematodes [10,11]. This is because mycorrhizal fungi encourage various defense mechanisms, including competition for photosynthates with pathogenic microbes and the expression of disease-resistance genes [12,13]. They also stimulate the expression of antioxidant enzymes and reduce malondialdehyde (MDA) production, an indicator of plant-membrane damage by lipid peroxidation [3]. Arbuscular mycorrhizal fungi influence the functioning of the root system, affect plant growth at different levels, and help reduce fertilizer input in the agricultural system without any significant yield loss [14,15]. Moreover, numerous studies have suggested that the AMF application in sunflower augmented its growth parameters and seed yield, even under biotic and abiotic stress conditions, via complex interactions with the host plant [16,17].

Plant growth-promoting rhizobacteria influence plant growth either directly by stimulating plant metabolism or by generating phytohormones such as auxins and gibberellins, solubilizing minerals, fixing atmospheric-nitrogen, or indirectly by mitigating detrimental effects of phytopathogenic microbes and improving stress tolerance. They also affect plant growth by increasing the availability of nutrients and reducing incidences of pathogenic infestation [18,19]. Further, inoculation with *P. fluorescens* may also accelerate seed germination, improve the emergence of seedlings, respond to various external stress factors, and enhance root structures [20,21]. Due to these properties, various inoculants of PGPR that tend to promote plant growth via one of the mechanisms (i.e., suppression of diseases in plants, improved absorption of nutrients, or production of phytohormones) are being commercialized. One such bacterial species is *Pseudomonas* which can be efficiently used as a bio-control agent for plants. The present study, therefore, aims to investigate the beneficial effects of AMF (*G. mosseae*) inoculation alone and in combination with PGPR (*P. fluorescens*) on the biochemical and morphological characteristics of sunflower and their possible use as biofertilizers.

2. Results

Significant variations were observed among all the treatments (<0.01) for all the characteristics analysed in the present study, except for harvest index and specific leaf weight (Table 1). The effects of *G. mosseae* and *P. fluorescens* inoculation on different morphological and biochemical characteristics of sunflower were investigated and recorded in Tables 2 and 3. Although all the treatments produced significant results, T6 was found to be the most efficient in enhancing the quality, as well as quantity, of the yield.

Table 1. Variance analysis of the effects of different treatments on the morphological and biochemical traits of sunflower.

Traits	Treatments	Residuals
df	5	10
Leaf length (cm)	172.947	1.814
F	95.35	
<i>p</i>	<0.001	
Leaf width (cm)	229.967	1.833
F	125.44	
<i>p</i>	<0.001	
Total yield (kg)	1.309	0.002
F	561.93	
<i>p</i>	<0.001	
Plant height (cm)	1764.668	24.361
F	72.44	
<i>p</i>	<0.001	
Plant weight (kg)	1.755	0.090
F	19.51	
<i>p</i>	<0.001	
Flower diameter (cm)	77.714	0.189
F	411.43	
<i>p</i>	<0.001	
Seed area (mm ²)	6224.013	109.649
F	56.76	
<i>p</i>	<0.001	
Seed length (mm)	97.014	7.087
F	13.69	
<i>p</i>	<0.001	
Seed width (mm)	16.426	0.520
F	31.59	
<i>p</i>	<0.001	
Seed per flower	199,304.722	2884.389
F	69.10	
<i>p</i>	<0.001	
×1000 SW (g)	7560.699	92.534
F	81.71	
<i>p</i>	<0.001	
Days to 50% flowering	9.600	0.333
F	28.80	
<i>p</i>	<0.001	
Days to maturity	12.400	0.133
F	93.00	
<i>p</i>	<0.001	
Head diameter (cm)	49.932	0.324
F	154.27	
<i>p</i>	<0.001	
No. of leaves per plant	40.161	0.439
F	91.41	
<i>p</i>	<0.001	
Specific leaf weight (g)	0.571	0.064
F	8.98	
<i>p</i>	0.015	

Table 1. Cont.

Traits	Treatments	Residuals
Relative leaf water content	175.977	8.314
F	21.17	
<i>p</i>	0.002	
Photosynthetic efficiency (SPAD reading)	18.506	0.296
F	62.55	
<i>p</i>	<0.001	
Seed yield per plant (g)	246.235	1.828
F	134.72	
<i>p</i>	<0.001	
Harvest Index	80.374	13.926
F	5.77	
<i>p</i>	0.039	
Oil content	12.113	0.181
F	67.09	
<i>p</i>	<0.001	
Palmitic acid	6.290	0.107
F	58.68	
<i>p</i>	<0.001	
Stearic acid	2.816	0.201
F	14.03	
<i>p</i>	0.006	
Oleic acid	118.098	0.651
F	181.32	
<i>p</i>	<0.001	
Linoleic acid	93.447	0.182
F	514.49	
<i>p</i>	<0.001	
Root colonization (%)	6558.210	2.351
F	1681.44	
<i>p</i>	<0.001	

Table 2. Effects of different treatments on various morphological characteristics of sunflower.

Traits	T1	T2	T3	T4	T5	T6
Leaf length (cm)	19.67 ± 1.15d	22.67 ± 2.31d	26.67 ± 0.58c	29.33 ± 0.58bc	32.50 ± 0.50b	41.00 ± 1.73a
Leaf width (cm)	15.67 ± 3.06d	21.00 ± 3.00c	26.33 ± 0.58b	29.33 ± 1.53b	36.00 ± 1.00a	38.67 ± 2.89a
Total yield (kg)	0.40 ± 0.02f	0.67 ± 0.09e	0.86 ± 0.09d	1.37 ± 0.13c	1.63 ± 0.10b	2.17 ± 0.14a
Plant height (cm)	71.79 ± 3.77c	80.56 ± 7.40c	90.71 ± 0.41c	119.17 ± 0.76b	123.90 ± 4.45b	149.21 ± 13.68a
Plant weight (kg)	0.25 ± 0.16c	0.46 ± 0.00c	0.52 ± 0.01c	0.88 ± 0.28bc	1.46 ± 0.37ab	2.27 ± 0.74a
Flower diameter (cm)	11.83 ± 0.29f	14.50 ± 1.00e	16.33 ± 0.58d	20.33 ± 0.58c	22.83 ± 1.26b	25.00 ± 1.00a
Seed area (mm ²)	93.90 ± 14.31d	148.23 ± 21.33c	176.03 ± 0.87bc	179.90 ± 0.96b	194.93 ± 12.15b	227.90 ± 24.34a
Seed length (mm)	10.87 ± 6.97b	21.10 ± 0.66a	23.00 ± 0.44a	24.20 ± 0.53a	25.03 ± 0.29a	26.70 ± 1.21a
Seed width (mm)	8.13 ± 0.81c	9.57 ± 0.23bc	10.00 ± 0.20bc	10.53 ± 0.32b	11.17 ± 0.21b	15.03 ± 1.94a
Seed per flower	488.33 ± 33.29e	709.00 ± 98.91d	821.33 ± 43.09cd	903.33 ± 22.48bc	1002.00 ± 48.14b	1244.33 ± 148.04a
X1000SW (g)	99.53 ± 24.20e	138.92 ± 12.76d	165.54 ± 10.21cd	181.06 ± 2.13bc	198.03 ± 8.22b	245.86 ± 20.45a
Days to 50% flowering	75.00 ± 0.00a	72.00 ± 1.41b	70.00 ± 0.00bc	70.00 ± 0.00bc	70.00 ± 0.00bc	69.00 ± 0.00c
Days to maturity	102.50 ± 0.71a	102.00 ± 0.00a	100.00 ± 0.00b	99.00 ± 0.00b	97.00 ± 0.00c	96.50 ± 0.71c
Head diameter (cm)	10.29 ± 1.71d	11.84 ± 0.06cd	13.58 ± 0.35c	17.34 ± 0.72b	20.84 ± 1.00a	22.62 ± 0.86a
No. of leaves per plant	16.89 ± 1.21e	19.64 ± 0.09de	21.02 ± 0.38cd	23.79 ± 0.76bc	26.10 ± 0.39b	29.16 ± 1.89a

Table 2. Cont.

Traits	T1	T2	T3	T4	T5	T6
Specific leaf weight (g)	0.75 ± 0.17b	1.06 ± 0.01b	1.14 ± 0.02b	1.33 ± 0.04ab	1.59 ± 0.13ab	2.29 ± 0.67a
Relative leaf water content	55.78 ± 4.04c	61.73 ± 2.00bc	64.44 ± 0.40bc	68.25 ± 1.18b	72.15 ± 0.55ab	82.92 ± 7.91a
Photosynthetic efficiency (SPAD reading)	32.22 ± 1.05d	34.47 ± 1.69cd	36.37 ± 0.28bc	37.95 ± 0.64ab	39.52 ± 0.34a	40.11 ± 0.42a
Seed yield per plant (g)	15.33 ± 1.41e	17.55 ± 1.04de	22.66 ± 2.81cd	28.27 ± 0.21c	36.15 ± 0.33b	43.92 ± 3.54a
Harvest Index	4.20 ± 0.17b	5.62 ± 0.43b	8.34 ± 1.25ab	10.93 ± 0.88ab	13.57 ± 0.02ab	21.61 ± 9.62a

Means ± SD followed by the same letters are not significantly different at $p < 0.05$ (Newman-Keuls test).

Table 3. Effects of different treatments on various biochemical characteristics and root colonization (%) of sunflower.

Traits	T1	T2	T3	T4	T5	T6
Oil content	26.59 ± 0.62c	27.77 ± 0.15c	28.23 ± 0.22c	30.14 ± 0.11b	31.80 ± 1.22ab	32.90 ± 0.28a
Palmitic acid	3.79 ± 0.08e	5.08 ± 0.83de	5.99 ± 0.23cd	6.69 ± 0.04bc	7.97 ± 0.02ab	8.51 ± 0.53a
Stearic acid	2.57 ± 1.02c	3.33 ± 0.06bc	3.66 ± 0.13bc	4.43 ± 0.04abc	4.97 ± 0.06ab	5.85 ± 0.85a
Oleic acid	40.06 ± 0.18d	41.69 ± 0.76d	49.23 ± 2.43c	52.82 ± 1.20b	56.62 ± 0.55a	58.67 ± 0.47a
Linoleic acid	31.82 ± 1.16e	33.98 ± 0.02d	37.72 ± 0.03c	39.05 ± 0.32c	46.77 ± 0.18b	48.89 ± 0.45a
Root colonization (%)	0.00 ± 0.00e	38.92 ± 4.18d	56.77 ± 1.90b	0.00 ± 0.00e	43.40 ± 5.91c	68.45 ± 3.18a

Means ± SD followed by the same letters are not significantly different at $p < 0.05$ (Newman-Keuls test).

2.1. Morphological Characteristics

Leaf length increased by 15% with T2, 35% with T3, 49% with T4, 65% with T5, and 108% with T6 as compared with T1; leaf width increased by 34% with T2, 68% with T3, 87% with T4, 130% with T5, and 147% with T6. The combined efficiency of AMF and PGPR for plant growth and development can further be confirmed with the increase in the number of leaves per plant, the maximum with T6 (73%) and minimum with T2 (16%), while T3, T4, and T5 produced a corresponding increase of 24%, 41%, and 55%, respectively. The leaves of the sunflower plant increased not only in number but also in terms of weight after inoculation, i.e., the specific leaf weight increased by 41%, 52%, 77%, 112%, and 205% with T2, T3, T4, T5, and T6, respectively. Inoculation with *G. mosseae* + *P. fluorescens* showed notable effects on plant height as well, where it produced progress of 12% with T2, 26% with T3, 66% with T4, 73% with T5, and 108% with T6. However, the most significant variations were recorded in plant weight where T6 produced a dramatic increase of about 808%, while T2, T3, T4, and T5 also produced remarkable increments of 84%, 108%, 252%, and 484%, respectively, as compared to T1. In addition, T6 also significantly enhanced flower characteristics with an increase of 111% in flower diameter, followed by 93% with T5, 72% with T4, 38% with T3, and 22% with T2. The head diameter also reported remarkable growths of 15% with T2, 32% with T3, 69% with T4, 103% with T5, and 120% with T6.

As far as the characteristics of seeds were concerned, the dual combination of *G. mosseae* and *P. fluorescens* was found to be the most effective rather than their separate applications. The seed area increased by 58%, 87%, 91%, 107%, and 143% respectively; seed length by 94%, 111%, 123%, 130%, and 146% respectively; and seed width by 18%, 23%, 29%, 37%, and 85% respectively after treatment with T2, T3, T4, T5, and T6. The number of seeds per flower exhibited a maximum increase with T6 (155%), followed by T5 (105%), T4 (85%), T3 (68%), and T2 (45%). In the case of seed yield, there were dramatic increments of 136% and 186% after treatment with T5 and T6; however, treatments T3 and T4 were also significant with an increase of 48% and 84%, respectively. Further, the increase of 39%, 66%,

82%, 99%, and 147% was observed in $\times 1000$ seed weight when subjected to treatments T2, T3, T4, T5, and T6, respectively. In addition to the upgraded structural features of different plant parts, the inoculation of *G. mosseae* and *P. fluorescens* also showed an improvement in total yield, which increased by 67% after treatment with T2, 115% with T3, 242% with T4, 307% with T5, and a maximum increase of 442% with T6. It is, however, worth mentioning here that the effects of *G. mosseae* and *P. fluorescens* inoculations on the number of days to 50% flowering did not show any significant variations, and exhibited only a nominal decrease of 4% with T2, 7% with T3, T4, T5 each, and 8% with T6. Similarly, the number of days to maturity remained almost the same with T2, decreased by 2% with T3, 3% with T4, 5% with T5, and 6% with T6. Furthermore, the harvest index also increased by 34%, 98%, 160%, 223%, and 415% after treatment of the plants with T2, T3, T4, T5, and T6, respectively. Furthermore, relative leaf water content was also found to be enhanced by 11%, 16%, 22%, 29%, and 49% with T2, T3, T4, T5, and T6, respectively. Regarding photosynthetic efficiency, it was observed that T2 (7%) and T3 (13%) showed minor differences. However, treatments T5 and T6 were quite efficient, displaying 23% and 24% increments, respectively.

2.2. Biochemical Characteristics

The oil content exhibited only a marginal increase of 4%, 6%, 13%, 20%, and 24% with T2, T3, T4, T5, and T6, respectively, which revealed that only a combined *G. mosseae* and *P. fluorescens* formulation could produce a notable increase in the sunflower oil content. Nevertheless, the symbiotic associations of AMF and PGPR with sunflower plants were also found to be favorable for its acid content, as palmitic acid increased by 34%, 58%, 77%, 110%, and 125%; stearic acid increased by 30%, 42%, 72%, 93%, and 128%; oleic acid by 4%, 23%, 32%, 41%, and 46%; and linoleic acid increased by 7%, 19%, 23%, 47%, and 54% after following the treatments T2, T3, T4, T5, and T6, respectively. No root colonization (%) was determined for the T1 and T4, respectively. At the same time, T2 (39.92%) and T3 (56.77%) showed less root colonization (%) under stress conditions as compared to the normal conditions T5 (43.40%) and T6 (68.45%), respectively.

3. Discussion

Results of the study as recorded in Table 2 revealed that AMF (*G. mosseae*) and PGPR (*P. fluorescens*) inoculation presented significant increments in all the growth parameters of sunflower plants. This may be attributed to the fact that these bioinoculants led to an increase in the surface area of roots, subsequently enhancing their water and mineral absorption efficiency, particularly slow dissolving mineral ions such as phosphorus, and resulting in better plant growth under water stress [22,23]. The reason might be the organic acids produced by plant growth-promoting micro-organisms, chelating the phosphate-bound cations, and increasing phosphorus accessibility in the soil [24,25]. The mycorrhiza hyphae extension can penetrate the soil pores which are inaccessible to root hairs subsequently absorbing more water than non-mycorrhizal plants. The improvements in plant growth parameters after AMF and PGPR inoculation were further supported by earlier experiments on sesame [26] and mustard [27,28].

In general, AMF inoculated sunflower plants possess larger leaves than non-inoculated plants as shown in Table 2. The photosynthetic rate associated with the chlorophyll content may be responsible for augmented leaf characteristics (leaf length and width), an increase in the number of leaves, and specific leaf weight [16,29]. Enhanced rate of water uptake, aided by AMF such as *G. mosseae* through upgraded hydraulic conductivity helps in increasing photosynthetic efficiency and consequently leaf area and leaf conductance [30,31]. These observations confirm the findings of other researchers, which showed an increased number of leaves per plant after AMF and PGPR inoculation in sunflower [32,33]. We observed the positive effects of *G. mosseae* and *P. fluorescens* inoculation on seed characteristics as shown in Table 2, which were also in agreement with the analysis of Arif et al. [34] which revealed that the inoculated sunflower plants had a significantly higher number of seeds per flower in addition to improved structural features, i.e., length, width, and area of

seed [34]. AMF also interacts with Rhizobacteria, which promotes plant growth. As PSB solubilizes sparingly accessible phosphorous compounds into orthophosphate, which AMF may absorb and transport to the host plant, AMF and phosphate solubilizing bacteria (PSB) could interact synergistically and improve plant health and productivity. Further, the study also indicated a significant increase in the yield of sunflower seeds. This may be explained by the fact that mycorrhizae symbiosis develops the photosynthetic source for plants through increased leaf area so that the crops might lead to a greater yield of seeds [35,36].

Furthermore, we also observed the augmented flower characteristics (flower diameter and head diameter) of sunflower after application of *G. mosseae* and *P. fluorescens*. However, the *G. mosseae* and *P. fluorescens* inoculations did not produce many variations in the number of days to 50% flowering and number of days to maturity (Table 2). The large size of flowers in AMF treated sunflower plants (which was again due to enhanced photosynthetic rate and increased photosynthate accumulation) subsequently leads to an increase in plant weight, [29,37] also observed in this investigation. The findings of other researchers also suggested a larger head diameter in AMF inoculated sunflower plants than in non-inoculated plants [33,38]. The increase in flower head diameter and the corresponding increase in plant weight, along with the increase in plant height can be ascribed to the improved uptake of some nutrients such as boron through root colonization with AMF. It should be noted that sunflower is considered sensitive to a lower boron supply, and deficiency may hinder its vegetative growth [39]. According to different studies, plant height, inter-nodal length, and flower head diameter increased significantly in sunflower plants with sufficient boron nutrition as compared to the control [40,41]. However, further investigations are required to confirm the significance of boron nutrition in other plant growth parameters. The increase in total yield, as per our observations, might also have resulted from improved root structure in inoculated sunflower plants leading to more water and nutrient absorption. The increase in the total yield of sunflower observed after AMF and PGPR application has been supported by numerous other studies on various crops such as switchgrass (*Panicum virgatum*) [42] and maize (*Zea mays*) [43,44].

Arbuscular mycorrhizal fungi pre-treated sunflower plants displayed more water absorption over other solutions of sugar, salt, kinetin, and salicylic acid [45]. Mechanisms significant for drought resistance in plants include reduced loss of water via transpiration, maintenance of membrane stability, and regulated osmotic adjustment. Application of *G. mosseae* enhances the root hydraulic conductivity at a low water potential of soil, which eventually influences the rate of transpiration, the resistance of leaves, and crop yield [46]. The increase in RLWC after *G. mosseae* inoculation also suggested more osmotic adjustment in the roots of inoculated plants at a low-water potential. Arbuscular mycorrhizal fungi are known to stimulate plant growth and produce plant hormones such as auxin, cytokinin, and gibberellin [47,48]. Furthermore, PGPR can significantly affect plant growth through increased nitrogen uptake, phytohormones synthesis, and mineral dissolution [49]. Inoculation with both AMF and PGPR thereby increased spore abundance, root colonization rate, and phosphorus content in tissues of sunflower at later stages of experiment [47,50,51]. Nevertheless, AMF species differ in the extent of benefits conferred by them on the development of host plants [36].

The higher colonization rates of *G. mosseae*, irrespective of water content in the soil, suggested greater adaptability of this species to native soils even under drought conditions. [52]. The present study also reported an increase in oil content under the influence of both *G. mosseae* and *P. fluorescens*. Any factor which is known to augment the biomass will improve the grain yield and eventually the oil yield owing to a higher rate of photosynthesis and increased photosynthates generation [53]. Majeed et al. [54] reported a substantial increase in the oil content of sunflower with the help of PGPR-based fertilizers [54]. Moreover, the fatty acid composition (palmitic acid, stearic acid, oleic acid, linoleic acid) of seed oil was also found to be enhanced significantly, as shown in Table 3. Owing to the beneficial effects of these bioinoculants on sunflower plants, it may be concluded that

G. mosseae and *P. fluorescens* provide a promising strategy to upgrade their structural and biochemical characteristics with low chemical input and result in a better crop yield.

4. Materials and Methods

4.1. Planting Material and Experimental Details

The experiment used a randomized complete block design with five replicates of each treatment to compare the findings using the popular hybrid PSH-996 (Punjab Agricultural University, Ludhiana, India). Polyhouse used in this study was settled in, Jharkhand, India (24°22'18" N, 86°19'27" E). There were 10 pots of every treatment. The soil was obtained from the experimental site and mixed in a 3:1 ratio with sand (soil:sand). Further, this mixture was sieved through a 2 mm sieve and autoclaved for two days at 121 °C for two h. Sunflower seeds were surface sterilized and grown in earthen pots in a polyhouse setting. Each pot was administered 10% of *G. mosseae* inoculum, either alone or in combination with *P. fluorescens*. The effect of these bio inoculants on various growth parameters of sunflower was determined under limited water conditions. The performance under reduced irrigations was determined by withholding irrigation during anthesis and soft dough crop growth stages [55]. Under normal conditions, six irrigations were applied during the crop season as suggested by Punjab Agricultural University, Ludhiana for a successful sunflower production under Northern Indian plains. Whereas in the treatments screened for water stress, only two irrigation schedules were performed during the crop season to generate a water stress environment as described in our previous work [55]. Hoagland's nutrient solution without P (100 mL/pot) was applied after every 15th day of transplantation [56]. A package of practices was followed for various agronomical practices as defined elsewhere [57].

4.2. Inoculation of AM Fungi and *Pseudomonas fluorescens*

Six treatments were evaluated in the experiment, i.e., T₁: control (drought-stressed plants), T₂: *G. mosseae* inoculation under drought stress, T₃: *G. mosseae* + *P. fluorescens* inoculation under drought stress, T₄: normal irrigation, T₅: *G. mosseae* inoculation under normal watering, T₆: *G. mosseae* + *P. fluorescens* inoculation under normal watering. For single AMF treatment, 100 g of *G. mosseae* was supplemented per pot. For *P. fluorescens* treatment, all the seeds were dipped in the nutrient broth medium for 10 min. In consortium treatment, both were applied. Overall, the experiment had six treatments, as follows:

- Control: T1
- G. mosseae*: T2
- G. mosseae* + *P. fluorescens*: T3
- Normal: T4
- G. mosseae*: T5
- G. mosseae* + *P. fluorescens*: T6

4.3. Plant Harvest and Analysis

Plants were harvested after 120 days of inoculation (DOI), and the effect of bioinoculants on various growth parameters was reported.

4.3.1. Morphological Characteristics

Plant height (cm) was recorded after 120 DOI by a 1 m scale from ground level to the head attachment of the plant (after attaining physiological maturity), and plant weight (kg) was measured using the weighing balance. Leaf length (cm) and leaf width (cm) were determined using a centimetre scale, and the number of leaves (per plant) was calculated as the average number of leaves at the stage of maturity. Specific leaf weight (g) (SLW) was measured as the total weight of the leaves divided by the number of plant leaves. The head diameter (cm) and flower diameter (cm) were recorded as the distance between two ends of the head and flower, respectively, at the time of maturity. The seed length (mm), seed width (mm), and seed area (mm²) were measured after harvesting the crop, and the

seed yield per plant (g) was determined as the average weight of the harvested seeds. The number of seeds per flower was determined by calculating the average value of the total number of seeds. Days to 50% flowering were counted from the sowing date until the opening of approximately 50% of the flower buds into flowers. Similarly, days to maturity were recorded from the sowing date until complete maturity (when the heads' backside turned brown). The harvest index (HI) was predicted at maturity using the formula $HI = 100 \times \text{seed yield} / \text{total biomass (seed yield + vegetative mass)}$.

4.3.2. Biochemical Characteristics and Root Colonization (%)

The relative leaf water content (RLWC) was estimated using 100 mg fresh weight of leaf discs, kept in distilled water till saturation. The leaf discs were taken out after 6 h, then water was blotted off from the surface of the discs without any pressure. The leaf discs were then weighed carefully to obtain a saturated weight. Finally, the dry weight was estimated after drying the leaf discs for 72 h at 70 °C. RLWC was then calculated using the formula, $RLWC = 100 \times ((\text{fresh weight} - \text{dry weight}) / (\text{saturated weight} - \text{dry weight}))$. The photosynthetic efficiency of leaves was measured using a soil plant analysis development (SPAD) meter as SPAD reading (SPAD 502 Plus Chlorophyll Meter, Minolta, Japan). The oil content (%) of sunflower seeds was determined with the help of a nuclear magnetic resonance (NMR) analyzer (Newport Analyzer MK III A, Newport Instruments Ltd., Milton Keynes, England). The standardization of NMR was performed with 4 g seeds of known oil content. The seed samples were cleaned and dried for 3 h at 11 °C in an oven and a representative sample of 2 g was then employed for oil content estimation. The oil content in each seed was calculated using the formula: $\text{CONSTANT} = \text{NMR reading of calibration standard} / (\text{weight of seed}) \times (\text{oil content of the standard, by extraction})$ and $\% \text{ OIL} = \text{NMR reading of seed} / (\text{weight of dry sample}) \times (\text{constant})$. The fatty acid composition (palmitic acid, stearic acid, oleic acid, linoleic acid) of sunflower oil was further determined using near-infrared (NIR) spectroscopy. The sunflower seeds were husked manually and their NIR spectra were scanned between 1100 to 2500 nm according to methods proposed by Sato et al. [58]. In order to assess the amount of root colonisation (percentage), the Phillips and Hayman staining technique [59] was used first, followed by the 'Giovannetti and Mosse' [60] method, both of which were carried out using a Lab Digital Trinocular Compound LED Microscope (Omax 40X-2500X, Kent, WA, USA). In order to calculate the proportion of AMF infected root segments, the following formula was used: $100 / (\text{number of root segments colonised} / \text{total number of root segments})$.

4.4. Data Analysis

Statgraphics Centurion XVI software was used for data analysis testing (StatPoint Technologies, Warrenton, VA, USA). A Newman-Keuls multiple-range test was used to statistically examine the findings, which were represented as mean values \pm standard deviation. The variance analysis and the Newman-Keuls multiple-range test were used to compute and statistically analyse the data acquired. In all cases, unless otherwise indicated, statistical significance was denoted by the value of $p < 0.05$.

5. Conclusions

Drought is a widespread adverse limiting factor that affects plant growth, physiology, and metabolism in different ways. Drought stress has an impact on the morphological, physiological, biochemical, and metabolic pathways of plants, resulting in a decrease in plant productivity. AMF and PGPR are vital in enhancing plant response to biotic and abiotic challenges, as well as reducing the consequences of these stresses on plants. Their role in enhancing plant growth and output, disease resistance, biotic and abiotic tolerance, and reducing the use of harmful pesticides and industrial fertilizers is an environmentally acceptable approach for reducing the use of pesticides and industrial fertilizers. Inoculation with mycorrhizal fungi and growth-promoting rhizobacteria can be considered efficient in augmenting growth parameters and total crop yield. Arbuscular mycorrhizal fungi

promote plant growth and crop yield by supplying the nutrients essential for the plants by developing an extra-radical hyphae network that increases the soil volume used by plant roots. Further, AMF root colonization helps in the absorption of nutrients and water from the soil, biocontrol of pathogens, production of plant hormones, and settlement of plantlets in unfavorable conditions.

Our experiment on sunflowers demonstrated distinct improvements in the quality and quantity attributes of almost all the plant parts including leaves, flowers, and seeds. All the morphological and biochemical parameters produced favorable results under integrated AMF and PGPR treatment, reflecting their efficiency to be used as biofertilizers. Further, it may be observed that bio-inoculation could be of much significance in water-stressed soils. The exogenic application of *G. mosseae* alone or with *P. fluorescens* alleviates the adverse effects of water stress on the sunflower yield and physiology. Moreover, inoculation of the studied micro-organisms *G. mosseae* and *P. fluorescens* are economically viable as well as sustainable, thus, they can be applied in fields for better growth and yield. Thus, we conclude that AMF and PGPR can possibly increase sunflower productivity while mitigating environmental degradation simultaneously.

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