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Enhancing sunflower growth and productivity through Arbuscular Mycorrhiza and *Pseudomonas fluorescens* Co-Inoculation: A sustainable agriculture approach

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Abstract
A field experiment was conducted during *Rabi* 2025-26 at Agricultural Research Station, Bheemarayanagudi, University of Agricultural Sciences, Raichur, Karnataka, to study the influence of arbuscular mycorrhiza and *Pseudomonas fluorescens* in combination with different levels of recommended dose of phosphorus on growth and seed yield of sunflower hybrid RSFH-700. The experiment was laid out in Randomized Complete Block Design with ten treatments and three replications. The treatments comprised control RDF, arbuscular mycorrhiza @ 2 kg ha^{-1}, *P. fluorescens* @ 4 g kg^{-1} seed and their combinations with 75 and 100 per cent recommended dose of phosphorus. Significant differences were observed among treatments for growth, flowering, yield attributes and seed yield. Treatment T_6, consisting of 100 per cent recommended dose of phosphorus + arbuscular mycorrhiza @ 2 kg ha^{-1}, recorded the highest plant height at harvest (136.3 cm), head diameter (17.9 cm), 100-seed weight (7.07 g), total seeds head^{-1} (828), filled seeds head^{-1} (736), seed filling (89.00 %), seed yield plant^{-1} (47.3 g) and seed yield (957.54 kg ha^{-1}). It also recorded the earliest flower bud initiation (50.0 days) and 50 per cent flowering (58.0 days). Treatment T_7, comprising 75 per cent recommended dose of phosphorus + *P. fluorescens* seed treatment @ 4 g kg^{-1} seed, recorded 134.8 cm plant height, 17.1 cm head diameter, 6.80 g hundred seed weight, 734 total seeds head^{-1}, 643 filled seeds head^{-1}, 87.67 per cent seed filling, 44.6 g seed yield plant^{-1} and 872.87 kg ha^{-1} seed yield and was on par with T_6 for major growth and yield traits. In contrast, T_{10}, consisting of 100 per cent recommended dose of phosphorus with both microbial inoculants, recorded comparatively lower values for several growth and yield attributes. The results indicated that arbuscular mycorrhiza with full recommended phosphorus or *P. fluorescens* with 75 per cent recommended phosphorus improved growth and seed yield of sunflower hybrid RSFH-700, with T_7 showing potential for reducing phosphorus fertilizer use without compromising major growth and yield performance.

Keywords: Arbuscular mycorrhiza, *Pseudomonas fluorescens*, phosphorus, seed yield and quality

Introduction
Modern agricultural systems face the critical challenge of maintaining high crop productivity while mitigating environmental degradation caused by the over application of synthetic chemical fertilizers. To establish ecologically sustainable farming models, modern agronomy must transition toward nature-based solutions that harness the latent potential of the plant rhizosphere. Soil microorganisms, particularly arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas fluorescens*, offer a promising, eco-friendly pathway to enhance plant fitness. These beneficial microbial symbioses function as biological fertilizers, improving soil structural health, enhancing nutrient cycling, and optimizing resource-use efficiency in ecologically vital crops like the sunflower (*Helianthus annuus*). By deciphering the synergistic dynamics between dual microbial co-inoculation and plant development, this study aims to provide a robust framework for reducing the ecological footprint of oilseed cultivation while safeguarding long-term agroecosystem resilience. (Saeedeh Rahimzadeh and Alireza Pirzad,2019) [8].

Sunflower (*Helianthus annuus* L.) is an important oilseed crop valued for its edible oil and adaptability to diverse agro-climatic conditions. In hybrid seed production, adequate vegetative growth, timely flowering, proper synchronization of parental lines and efficient seed filling are important for obtaining higher quantities of quality hybrid seed. Phosphorus plays an important role in root development, energy transfer, flowering and reproductive development. However, the efficiency of applied phosphorus may be influenced by soil properties and nutrient availability.

Arbuscular mycorrhizal fungi form symbiotic associations with plant roots and can improve nutrient acquisition, particularly phosphorus, through expansion of the effective soil-root interface. Similarly, phosphate-solubilizing plant-growth-promoting bacteria such as *Pseudomonas* can contribute to phosphorus availability and plant growth through phosphate solubilization and other growth-promoting mechanisms (Rodríguez and Fraga, 1999) [6]. Previous studies in sunflower have reported beneficial effects of mycorrhizal inoculation and phosphorus application on growth, yield attributes and seed yield

(Soleimanzadeh, 2010; Kalaiyarasan *et al*., 2017) [3, 9]. Co-inoculation of AMF and *Pseudomonas* has also been investigated in sunflower under different phosphorus regimes (Babaei *et al*., 2012) [1].

Although microbial inoculants can improve nutrient acquisition, their response may depend on the level of externally supplied phosphorus and the interaction among microbial treatments. Therefore, the present investigation was undertaken to evaluate the effect of arbuscular mycorrhiza and *Pseudomonas fluorescens* in combination with different phosphorus levels on growth and seed yield of sunflower hybrid RSFH-700 under hybrid seed production conditions.

Materials and Methods

The field experiment was conducted during *Rabi* 2025-26 at Agricultural Research Station, Bheemarayanagudi, University of Agricultural Sciences, Raichur, Karnataka. The experimental field was characterized by medium-deep black soil with a soil pH of 8.3. The experiment was conducted with planting ratio 3:1, female parent CMS-38A and male parent RGM-49 of sunflower hybrid RSFH-700. The experiment consisted of ten treatments replicated three times in Randomized Complete Block Design. The gross plot size was 4.8 × 3.0 m. An isolation distance of 400 m was maintained to avoid contamination. Two seeds were dibbled per hill at 60 × 30 cm spacing. Female parent CMS-38A was sown two days before the male parent RGM-49 to obtain flowering synchrony. The recommended fertilizer dose was 90:90:60 kg N: P_2O_5: K_2O ha^{-1}.

The treatment was as follows:

T_1	Control RDF
T_2	Arbuscular mycorrhiza @ 2 kg ha^{-1} soil application
T_3	*P. fluorescens* seed treatment @ 4 g kg^{-1} seed
T_4	Arbuscular mycorrhiza @ 2 kg ha^{-1} + *P. Fluorescens* seed treatment@ 4 g kg^{-1} seed
T_5	75% RDP + arbuscular mycorrhiza @ 2 kg ha^{-1}
T_6	100% RDP + arbuscular mycorrhiza @ 2 kg ha^{-1}
T_7	75% RDP + *P. fluorescens*seed treatment@ 4 g kg^{-1} seed
T_8	100% RDP + *P. fluorescens*seed treatment@ 4 g kg^{-1} seed
T_9	75% RDP + arbuscular mycorrhiza + *P. fluorescens*seed treatment@ 4 g kg^{-1} seed
T_{10}	100% RDP + arbuscular mycorrhiza + *P. fluorescens*seed treatment@ 4 g kg^{-1} seed

Pseudomonas fluorescens was applied as a seed treatment at 4 g kg^{-1} seed and the treated seed was shade dried before sowing. Arbuscular mycorrhiza was applied to the soil at 2 kg ha^{-1} one day after sowing. The microbial treatments were imposed on both parental lines according to the respective treatment. Supplementary pollination was performed for 15 days. by collecting pollen from the male parent and applying it to the corresponding female parent. At maturity, male plants were harvested first followed by female plants. Heads were shade dried, threshed and cleaned before recording seed yield. The observation was recorded on plant height, Days to flower bud initiation and days to 50 per cent flowering, head diameter, total seeds head^{-1}, filled seeds head^{-1}, seed filling percentage, 100-seed weight, and seed yield ha^{-1}.(Table 1).

Results and Discussion

Plant growth

The treatments significantly influenced plant growth of sunflower hybrid RSFH-700. At 60 DAS the highest plant height recorded in T_6 (100 % RDP + arbuscular mycorrhiza) recorded 109.2 cm followed by T_7 recorded 105.0 cm and T_{10} recorded the lowest plant height (76.1 cm). At harvest T_6 recorded the highest plant height at harvest (136.3 cm), followed by T_7 (75% RDP + *P. fluorescens*) with 134.8 cm. The superior growth under T_6 may be related to improved phosphorus acquisition through the mycorrhizal association. AMF can increase the effective absorbing area of roots and facilitate acquisition of relatively immobile nutrients such as phosphorus. Similar enhancement of sunflower growth and yield through VAM inoculation and phosphorus application has been reported by Kalaiyarasan *et al*. (2017) and Premi *et al*. (2012) [3, 5]. Recent field research has likewise demonstrated positive agronomic responses of sunflower to AM fungal inoculation. The performance of T_7 was particularly notable because it produced plant height comparable to T_6 with 25 per cent lower phosphorus application. The response may be associated with the ability of *P. fluorescens* to solubilize unavailable forms of phosphorus and promote plant growth. Phosphate-solubilizing bacteria are known to enhance phosphorus availability through mechanisms including organic-acid production and phosphatase activity (Rodríguez and Fraga, 1999) [6].

Flowering behaviour

The treatment T_6 recorded the earliest flower bud initiation (50.0 days), which was on par with T_7 (50.3 days). T_{10} recorded the latest flower bud initiation (54.0 days). For 50 per cent flowering, T_6 recorded 58.0 days followed by T5 and T_7, whereas T_{10} required 61.33 days. The earlier reproductive transition under T_6 may be associated with improved phosphorus availability and plant nutritional status. Phosphorus is important in energy metabolism and reproductive development, and adequate phosphorus availability can support the transition from vegetative to reproductive growth. The response observed with T_7 indicates that microbial phosphorus mobilization may partially compensate for reduced external phosphorus supply (Borjian *et al*., 2025) [2].

Head diameter

The largest head diameter (17.9 cm) was recorded in the treatment T_6, followed by T_7 (17.1 cm). The smallest head diameter (13.9 cm) was recorded in T_{10}. The greater head diameter under T_6 may have resulted from better vegetative growth and phosphorus nutrition, which subsequently supported capitulum development. Previous sunflower studies have also demonstrated improved growth and yield attributes with mycorrhizal inoculation and phosphorus application (Soleimanzadeh, 2010 [9] and Kalaiyarasan *et al*., 2017). Soleimanzadeh (2010) [3, 9] reported that mycorrhizal inoculation improved head diameter, number of seeds head^{-1} and seed yield, while the response to mycorrhiza varied with phosphorus level.

100-seed weight

The treatment T_6 recorded the highest 100-seed weight of 7.07 g and was statistically comparable with T_7 (6.80 g), T_5 (6.75 g), T_8 (6.69 g) and T_4 (6.59 g). The lowest value was

recorded in T_{10} (6.03 g). The higher seed weight under T_6 may be attributed to improved phosphorus availability and more efficient assimilate production during seed development. Mycorrhizal inoculation has been reported to improve seed yield and related traits in sunflower, particularly when combined with adequate phosphorus. (Rodríguez and Fraga, 1999) [6]

Number of seeds and filled seeds per head

Treatment T_6 recorded the maximum total number of seeds head^{-1} (828), followed by T7 (734). T_{10} recorded the lowest number (556). For filled seeds, T_6 recorded 736 seeds head^{-1}, followed by T_7 with 643; whereas T_{10} recorded 445 filled seeds. The higher number of filled seeds under T_6 may be associated with improved phosphorus availability during reproductive development. Phosphorus is involved in energy transfer and assimilate partitioning, which are important for reproductive growth and seed formation. Similar positive responses of sunflower to mycorrhizal inoculation and phosphorus have been reported by Kalaiyarasan *et al.* (2017) [3].

Seed filling (%)

The highest seed filling percentage (89.00%) was recorded in treatment T_6, followed by T_7 (87.67%). The control recorded 83.00 per cent, whereas T_{10} recorded the lowest seed filling (80.00%). The higher seed filling under T_6 indicates that adequate phosphorus supply together with mycorrhizal inoculation supported reproductive development and assimilates partitioning. (Tewari and Arora, 2014) [10].

Seed yield per hectare

The treatment T_6 recorded the highest seed yield of 957.54 kg ha^{-1}, followed by T_7 with 872.87 kg ha^{-1} and T_5 recorded 862.47 kg ha^{-1}. The control treatment recorded 780.77 kg ha^{-1}, whereas T_{10} recorded the lowest seed yield of 712.74 kg ha^{-1}. The superiority of T_6 was associated with its favourable performance for plant height, head diameter, seed number, seed filling and 100-seed weight. The performance of T_7 was particularly important because it was statistically on par with T_6 for the major growth and yield traits despite receiving 25 per cent less recommended phosphorus (Mahanta *et al.*, 2014) [4]. The beneficial response of microbial inoculation is supported by earlier studies. Babaei *et al.* (2012) [1] evaluated mycorrhiza, *P. fluorescens* and phosphorus in sunflower and reported significant effects of phosphorus and mycorrhiza on several agronomic traits. Sharma *et al.* (2021) [7] also reported beneficial effects of AMF and *P. fluorescens* inoculation on sunflower growth and yield under water-stress conditions. (Table 1 and Fig. 1)

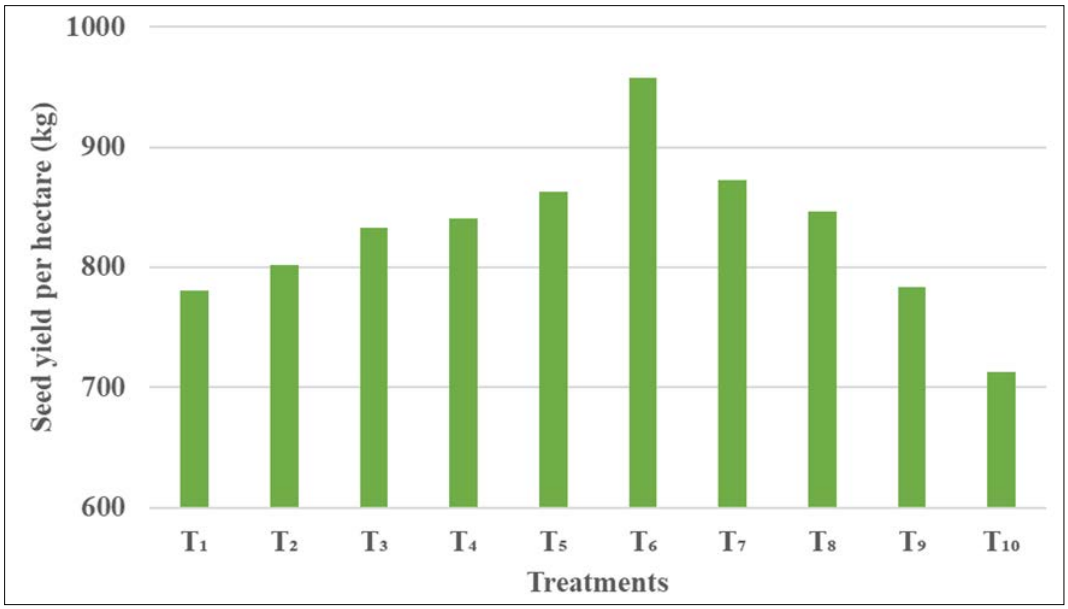


Fig 1: Effect of arbuscular mycorrhiza and *Pseudomonas fluorescens* on seed yield per hectare of sunflower hybridRSFH-700

Table 1: Effect of arbuscular mycorrhiza and *Pseudomonas fluorescens* on growth and yield parameters of sunflower hybrid RSFH- 700

Treatments	Plant height (cm) at		Days to bud initiation	Days to 50 % flowering	Head diameter (cm)	Test weight (g)	Total no. of seeds/ head	No. of filled seeds/ head	Seed filling (%)	Seed yield per hectare (kg)
	60 DAS	harvest								
T_1 - Control RDF	80.2	128.3	53.7	60.33	15.2	6.29	604	501	83.00	780.77
T_2 - Arbuscular mycorrhiza @ 2 kg/ha soil application	86.7	128.3	53.0	59.33	16.0	6.32	627	525	84.00	801.57
T_3 -*Pseudomonas fluorescens* seed treatment @ 4 g kg^{-1} seed	89.2	130.7	52.3	59.00	16.0	6.40	632	533	84.33	832.76
T_4- arbuscular mycorrhiza @ 2 kg/ha soil application + *Pseudomonas fluorescens* seed treatment @ 4 g kg^{-1} seed	93.2	130.9	52.0	59.00	16.1	6.59	640	541	84.67	840.19
T_5 - 75 % RDP + arbuscular mycorrhiza @2kg /ha soil application	104.2	132.1	51.0	58.33	16.6	6.75	720	617	85.67	862.47
T_6 -100 % RDP + arbuscular mycorrhiza @ 2 kg/ha soil application	109.2	136.3	50.0	58.00	17.9	7.07	828	736	89.00	957.54

T_7 - 75 % RDP + *Pseudomonas fluorescens* seed treatment @ 4 g kg^{-1} seed	105.0	134.8	50.3	58.33	17.1	6.80	734	643	87.67	872.87
T_8 – 100 % RDP + *Pseudomonas fluorescens* seed treatment @ 4 g kg^{-1} seed	97.1	131.0	51.3	59.33	16.1	6.69	652	555	85.00	846.13
T_9- 75 % RDP + arbuscular mycorrhiza @ 2 kg/ha soil application+ *Pseudomonas fluorescens* seed treatment @ 4 g kg^{-1} seed	83.2	129.4	53.3	59.33	15.3	6.29	607	508	83.67	783.74
T_{10}-100 % RDP + arbuscular mycorrhiza @ 2 kg/ha soil application + *Pseudomonas fluorescens* seed treatment @ 4 g kg^{-1} seed	76.1	126.3	54.0	61.33	13.9	6.03	556	445	80.00	712.74
Mean	92.4	130.8	52.1	59.23	16.0	6.52	660	561	84.70	829.08
S.Em.±	4.32	1.81	0.19	0.55	0.68	0.18	42	35	0.89	31.3
CD @ 5%	12.95	5.42	0.56	1.65	2.05	0.54	126	104	2.68	92.9

Conclusion

The investigation demonstrates that the targeted management of rhizosphere interactions using dual microbial inoculants-arbuscular mycorrhizal fungi (AMF) and *Pseudomonas fluorescens*-exerts a profound regulatory influence on the growth, floral phenology and reproductive fitness of *Helianthus annuus*. While the maximum absolute seed yield (957.54 kg ha^{-1}) was recorded under full chemical phosphorus allocation paired with soil-applied AMF (T_6), the most ecologically significant breakthrough emerged from the reduced-input regimen (T_7). Integrating a 75% recommended phosphorus dose with a *P. fluorescens* seed treatment (4 g kg^{-1}) yielded vegetative and reproductive outcomes statistically on par with the higher chemical fertilizer treatment.

These findings indicate that bio-fertilisation via beneficial soil microbiomes can successfully offset a 25% reduction in synthetic mineral inputs without jeopardising oilseed crop productivity. From an environmental management perspective, adopting such microbial co-inoculation strategies offers a viable pathway for the optimisation of nutrient-use efficiency, the minimisation of agricultural runoff and the advancement of sustainable agroecosystem conservation.

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