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The Effects of Phosphorus and Mycorrhizal Applications on Dry Matter Yield, Phosphorus Uptake, and Cotton Root and Shoot Morphology

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Abstract

Phosphorus (P) availability and mycorrhizal connections are critical in increasing agricultural production by improving nutrient absorption and stimulating plant development. This study looks at the interactions between phosphorus treatment and mycorrhizal inoculation on cotton (*Gossypium hirsutum* L.) in a controlled greenhouse environment. The experiment had two phosphorus levels (0 mg P_2O_5 kg^{-1} and 50 mg P_2O_5 kg^{-1}) and three mycorrhizal treatments: control (no inoculation), indigenous mycorrhiza, and *Glomus clarum*. Plant height, stem diameter, root and shoot morphology, dry matter yield (DW), and phosphate absorption were all evaluated thoroughly. In addition, Mycorrhizal Dependency (MD) was measured to determine the level of plant reliance on mycorrhizal connections. The study found that combining phosphorus with mycorrhizal inoculation significantly boosted several growth indices, including root shape, stem diameter, and dry matter production. *Glomus clarum* mixed with phosphorus treatment had the highest dry matter production (47.38 g pot^{-1}). Mycorrhizal reliance was discovered to be inversely related to phosphorus availability, with non-phosphorus-treated pots having higher dependency (11.3%) than phosphorus-treated pots (8.1%). These findings emphasize the synergistic effects of combining phosphate delivery with mycorrhizal inoculation to boost cotton growth, particularly in phosphorus-deficient soils. The study sheds light on sustainable agricultural techniques, particularly the potential of mycorrhizal fungi as bio-inoculants for optimizing nutrient management and increasing crop yields.

Keywords: *Phosphorus application, Mycorrhiza, Gossypium hirsutum, Dry matter yield, Nutrient uptake, Sustainable agriculture*

Introduction

As the main raw material for the textile industry, cotton (*Gossypium hirsutum* L.) is essential to the world economy. Its versatility is demonstrated by the fact that it is widely grown in a variety of agroecological zones; yet, phosphorus (P) deficiency continues to be a significant obstacle to reaching maximum yield. Energy transmission, nucleic acid synthesis, photosynthesis, and root growth in plants all depend on phosphorus, a crucial macronutrient (Smith et al., 2021). Phosphorus plays a vital function, but its availability in agricultural soils is frequently restricted because it is converted into forms that are insoluble and so unavailable to plant roots.

As a result, there is now a great dependence on synthetic phosphorus fertilizers, which raises production costs and presents serious environmental problems such soil deterioration, eutrophication, and groundwater pollution (Wang et al., 2023). Arbuscular mycorrhizal fungi (AMF) are a new sustainable way to alleviate phosphorus deficit and lessen reliance on artificial fertilizers. By spreading their hyphal networks into the soil, these symbiotic microbes establish mutualistic relationships with plant roots and mobilize phosphorus reserves that would otherwise be unavailable (Berruti et al., 2016). AMF contributes to greater resilience and general plant health by improving plant tolerance to biotic and abiotic stimuli in addition to increasing phosphorus absorption (Wu et al., 2020).

Glomus clarum is one of the AMF species that has drawn the most attention due to its exceptional capacity to enhance phosphorus uptake and increase crop yield (Lee et al., 2022). According to recent research, soil nutrient levels affect how well AMF promotes plant development, with phosphorus availability being a key factor. Research on the relationship between phosphorus treatment and certain AMF species is still ongoing, particularly in crops like cotton that require a lot of nutrients. The combined effects of AMF inoculation and phosphorus supplementation on root and shoot shape, phosphorus absorption, and biomass production are still poorly understood, despite some studies showing notable gains in cotton growth under mycorrhizal inoculation (Smith et al., 2022).

This study examines how AMF inoculation and phosphorus treatment work together to promote cotton growth in a controlled greenhouse environment. It specifically looks at how *Glomus clarum* and native mycorrhizae enhance dry matter output, phosphate absorption, and root and shoot morphological characteristics. The goal of this research is to better understand these interactions in order to guide the creation of integrated nutrient management plans that maximize cotton output in a sustainable manner by combining chemical and biological inputs.

2. Materials and Methods

To reduce external variability and assess the direct effects of mycorrhizal inoculation and phosphorus (P) treatment on cotton development, the experiment was carried out in a controlled greenhouse. Phosphorus levels (0 mg P_2O_5 kg^{-1}, designated as P0, and 50 mg P_2O_5 kg^{-1}, designated as P50) and three mycorrhizal treatments (control with no inoculation, indigenous mycorrhiza [IM], and *Glomus clarum* inoculation) were the two factors in a factorial experimental design that was carried out within a completely randomized design

(CRD). Three duplicates of each treatment combination were made. The impact of P supplementation and mycorrhizal colonization on plant growth metrics, root morphology, nutrient absorption, and biomass production could be thoroughly examined thanks to this methodology.

The growth medium had a pH of 7.2, an organic carbon content of 1.5%, and an accessible phosphorus concentration of 8 mg kg^{-1}. It was sandy loam soil. Autoclaving was used to sterilize the soil for two days in a row at 121°C in order to eradicate native microbial populations. The soil was completely homogenized after sterilizing, and to guarantee even distribution, phosphorus was added as triple superphosphate (TSP) prior to planting.
Sterilized soil mixture was poured into each 5-kg pot. During the sowing process, 10 g of mycorrhizal inoculum per pot was added to the rhizosphere. A combination of spores, hyphae, and root fragments colonized with *Glomus clarum* or indigenous mycorrhiza (IM) made up the inoculum, which ensured efficient colonization throughout plant growth.

Before being sown, cotton seeds (*Gossypium hirsutum* L.) were thoroughly washed with deionized water and surface-sterilized using a 0.1% sodium hypochlorite solution. Each container contained three seeds, which were then thinned to one plant upon germination. The plants were kept in a greenhouse with a 14-hour photoperiod, a regulated temperature (25 ± 2°C), and a relative humidity of 60–70%. Throughout the trial, no extra nutrients were given, and pots were watered often to maintain field capacity.

A tape and digital vernier caliper were used to measure the height of the plants and the diameter of their stems once a week. After the roots were completely cleaned, morphological characteristics such as volume, surface area, average diameter, and total length were measured. The effects of mycorrhizal and phosphorus inoculation on root development were examined by manually measuring and quantifying the cleaned roots. Understanding how treatments affected root architecture is essential for comprehending nutrient uptake and the potential for plant development.

After being collected individually, the shoot and root tissues were coarsely crushed and oven-dried to a consistent weight at 70°C. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was used to measure the amounts of phosphorus after acid digestion using a 4:1 v/v combination of nitric and perchloric acids. Tissue phosphorus concentrations were multiplied by the respective dry weights to determine total phosphorus intake.

Plants were collected and divided into shoots and roots at the conclusion of the experiment. The dry matter yield (DMY) was measured after each component was oven-dried at 70°C until a consistent weight was achieved. The total dry weight of the shoots and roots in each pot was used to compute the total DMY.

The statistical program JMP8 was used to examine the data. The effects of mycorrhizal treatments, phosphorus levels, and their interactions on the parameters that were evaluated were assessed using two-way analysis of variance (ANOVA). At a significance threshold of $p < 0.05$, treatment means were separated using Fisher's Least Significant Difference (LSD) test. Data were examined for homogeneity of variances using Levene's test and for normality using the Shapiro-Wilk test before analysis.

3. Results

3.1 Morphological Parameters

Morphological features, which represent the interaction of genetic potential and environmental factors, are important markers of plant health and vigor. In this study, stem diameter showed substantial responses to phosphate and mycorrhizal applications, but plant height did not significantly vary between treatments. The greatest stem diameters were produced by treatments that combined *Glomus clarum* with phosphorus application (P50), suggesting better resource allocation and potentially more robust structural support to maintain improved growth dynamics. The increase in stem diameter under these treatments is consistent with mycorrhizal fungi's capacity to enhance water and nutrient absorption (Berruti et al., 2016) and phosphorus' known function in encouraging cell division and elongation (Smith et al., 2021). Additionally, the combined application of phosphorus and mycorrhizal inoculation may have enhanced metabolic efficiency, which has been linked to the observed morphological alterations. This has been confirmed in previous research on cotton and other crops (Lee et al., 2022; Wu et al., 2020). These results highlight how combining these treatments might help cotton farmers get the best possible structural and functional development. There were no discernible variations in plant height across treatments (Table 1). *Glomus clarum* + P50 produced the highest diameter, although P and mycorrhizal treatments significantly increased stem diameter (Figure 1).

3.2 Dry Matter Yield

The DW yields for each treatment varied significantly (Table 2). The indigenous mycorrhiza (IM) + P50 treatment yielded 45.1 g pot^{-1}, whereas the *Glomus clarum* and 50 mg P_2O_5 kg^{-1} (P50) combination gave the greatest overall DW production of 47.38 g pot^{-1}. These findings suggest that mycorrhizal and phosphorus inoculation work in concert, most likely as a consequence of increased nutrient intake and metabolic efficiency. Studies by Wang et al. (2023) and Lee et al. (2022) that documented notable biomass gains in phosphorus-supplemented crops colonized by effective mycorrhizal species are consistent with the higher DW yield in mycorrhizal treatments.

The results emphasize the importance of microbial compatibility and soil conditions in impacting treatment effectiveness, which is in contrast to findings in some non-responsive soils where mycorrhizal inoculation had minimal effect (Wu et al., 2020). The considerably higher DW yield with *Glomus clarum* + P50 further suggests that species-specific colonization efficiency is essential for biomass accumulation, which is consistent with recent studies showing *Glomus* species' superior adaptability in nutrient-deficient environments (Smith et al., 2022). These findings demonstrate that in order to boost agricultural yields, tailored nutrient and mycorrhizal management strategies are required. The DW yields showed significant differences. IM + P50 (45.1 g pot^{-1}) had the second-highest total DW, while *Glomus clarum* + P50 (47.38 g pot^{-1}) had the highest.

3.3 Phosphorus Uptake

Figure 2 and Table 2 demonstrate that the combined treatments of phosphorus administration and mycorrhizal inoculation had a considerable impact on phosphorus absorption. The *Glomus clarum* + P50 therapy had the maximum P uptake, demonstrating the complementary nature of both treatments. The vast hyphal networks created by mycorrhizal fungi, which expand the root surface area and provide access to soil P fractions that plants would not otherwise be able to reach, are the mechanism behind the increased P absorption. This is consistent with research by Smith et al. (2022), which showed that *Glomus* species are especially effective in mobilizing rhizosphere-based labile and non-labile P fractions.

P-treated pots had greater P concentrations in both roots and shoots than non-P-treated pots, according to the results, which also show a difference absorption efficiency. According to Wang et al. (2023), this pattern emphasizes how phosphorus drives mycorrhizal efficiency. Additionally, the P50 treatments' increased P allocation to above-ground biomass points to increased metabolic activity, supporting research by Lee et al. (2022) and Berruti et al. (2016). Together, our results highlight how crucial it is to combine targeted mycorrhizal inoculation with phosphorus administration to maximize fertilizer usage efficiency in cotton farming. Mycorrhizal and P-treated plants exhibited a markedly increased absorption of phosphorus (Figure 2, Table 2). The greatest P absorption was seen in *Glomus clarum* + P50.

3.4 Mycorrhizal Dependency

Mycorrhizal Dependency (MD) was found to be substantially greater in pots without phosphorus (P) treatment (11.3%) than in pots treated with P (8.1%). This discovery emphasizes the compensatory role of phosphorus application in plant nutrient acquisition, as the presence of P from external sources lessens the plant's reliance on mycorrhizal fungi for nutrient uptake. The findings highlight the dynamic interplay between soil nutrient additions and symbiotic partnerships, in which increased P availability reduces the functional relevance of mycorrhizal associations in supplying plant phosphorus requirements."

4. **Discussion**

The findings of this study highlight the critical role that mycorrhizal inoculation and phosphorus (P) treatment play in improving cotton growth and nutrient uptake, particularly phosphorus, which is frequently a limiting resource in agricultural systems. Mycorrhizal fungi have long been known to increase plant nutrition by increasing root surface area and enabling the uptake of vital nutrients, particularly in nutrient-deficient soils (Smith et al., 2021). This study supports such findings, as *Glomus clarum* regularly outperformed indigenous mycorrhizal (IM) treatments in terms of increasing dry matter (DW) yield and phosphate uptake. *Glomus* *clarum's* improved performance can be due to its greater colonization efficiency and ability to mobilize phosphorus from both labile and non-labile pools in soil (Berruti et al., 2016; Smith et al., 2022). This species is extremely adept at obtaining phosphate fractions that would otherwise be unavailable to plants, enhancing its capacity to increase plant growth under phosphorus-limited circumstances. Such findings are consistent with prior research demonstrating how mycorrhizal fungi, particularly *Glomus* species, are effective in extending root systems into deeper soil layers and allowing plants to absorb phosphorus that would otherwise be unavailable (Berruti et al., 2016; Smith et al., 2022).

The synergistic impact shown between mycorrhizal inoculation and phosphorus administration in this study demonstrates the possibility for combining both treatments to increase cotton productivity. Combining *Glomus clarum* with 50 mg P_2O_5 kg^{-1} (P50) led in the best total dry matter yield and phosphorus uptake, highlighting the complimentary effects of these two therapies. Phosphorus is known to be an essential nutrient for plant development, and its availability has a substantial impact on plant growth, particularly in crops with high phosphorus requirements, such as cotton (Wang et al., 2023). The addition of phosphorus boosts the plant's nutrient supply, whereas the mycorrhizal inoculant improves the plant's ability to absorb and utilize phosphorus from the soil. This dual method not only enhances phosphorus efficiency but also minimizes the requirement for large phosphorus inputs, which are frequently expensive and environmentally harmful (Wu et al., 2020). The synergistic effect of these two treatments demonstrates the potential for more environmentally friendly farming techniques that reduce reliance on chemical fertilizers while improving nutrient uptake and crop output.

Recent investigations have confirmed the efficiency of combining mycorrhizal inoculation and phosphorus administration in boosting crop production. Lee et al. (2022) found that cotton plants inoculated with effective mycorrhizal species like *Glomus clarum* produced more biomass and absorbed more nutrients when fertilized with phosphate. Similarly, Wang et al. (2023) discovered that combining mycorrhizal inoculation with phosphorus administration greatly enhanced cotton dry matter production and phosphorus uptake, with mycorrhizal fungi playing an important role in soil nutrient mobilization. These findings are congruent with those of this study, which found that combining *Glomus clarum* and phosphorus greatly increased both plant growth and nutrient uptake when compared to phosphorus-only or mycorrhizal-only treatments. *Glomus clarum* outperforms indigenous mycorrhizal species (IM) due to its higher colonization efficiency and ability to mobilize phosphorus in nutrient-deficient environments, as observed in similar studies (Berruti et al., 2016; Smith et al., 2022).

The idea of mycorrhizal dependency (MD), which refers to how much plants rely on mycorrhizal fungi for nutrient uptake, was also investigated in this study. The results showed that the MD was higher in non-phosphorus-treated pots (11.3%) than in phosphorus-treated pots (8.1%), implying that applying phosphorus reduced the plant's reliance on mycorrhizal fungus for phosphorus uptake. This finding is consistent with prior research by Smith et al. (2021), who discovered that when plants receive phosphorus through fertilization, they become less reliant on mycorrhizal fungus for nutrient acquisition. Plants in phosphorus-deficient soils, on the other hand, frequently demonstrate increased mycorrhizal reliance, as fungi play an important role in mobilizing phosphorus from the soil (Wang et al., 2023). This study demonstrates that phosphorus treatment reduces the need for mycorrhizal fungi to some extent; yet, in the absence of phosphorus fertilization, mycorrhizal inoculation becomes an important element in guaranteeing appropriate phosphorus uptake.

This study's findings have important implications for the sustainability of cotton production. Phosphorus is a nonrenewable resource that is becoming increasingly scarce in global agriculture as a result of overexploitation and depletion of phosphate sources. Therefore, improving the efficiency of phosphorus use in crops through integrated approaches such as mycorrhizal inoculation and phosphorus fertilization can help reduce reliance on chemical fertilizers, lower production costs, and mitigate the environmental impact of phosphorus runoff (Lee et al., 2022). Furthermore, this strategy can improve soil health by encouraging beneficial soil microbes, lowering nutrient leaching, and increasing long-term sustainability in agricultural systems (Wu et al., 2020). As global cotton demand rises, optimal nutrient management options, such as integrating mycorrhizal fungi and phosphorus, will be important to attaining productivity targets while reducing cotton farming's environmental footprint.

5. Conclusion

Finally, this research supports the favorable benefits of mycorrhizal inoculation, notably with *Glomus clarum*, in increasing cotton growth and phosphorus uptake, especially when paired with phosphate fertilization. The combination of these two treatments not only improves phosphorus efficiency, but it also encourages sustainable farming practices by lowering the need for excessive phosphorus fertilization. Future study should focus on optimizing mycorrhizal and phosphorus management tactics across a variety of soil types and environmental situations, as well as investigating the long-term effects of these treatments on soil health and crop productivity.

6. **Acknowledgment**

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7. References

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Table 1. Plant Height and Stem Diameter Across Treatments

Treatment	Plant Height (cm)	Stem Diameter (mm)
Control + P0	50.2 ± 2.1	3.2 ± 0.2
Control + P50	51.8 ± 1.8	3.5 ± 0.3
IM + P0	52.0 ± 2.0	4.0 ± 0.2
IM + P50	53.5 ± 1.5	4.3 ± 0.2
Glomus clarum + P0	54.0 ± 2.3	4.5 ± 0.1
Glomus clarum + P50	55.3 ± 1.7	4.8 ± 0.3

Table 2. Phosphorus Uptake Across Treatments

Treatment	Shoot P Content (mg kg^{-1})	Root P Content (mg kg^{-1})	Total P Uptake (mg pot^{-1})
Control + P0	12.1 ± 0.6	8.3 ± 0.4	20.4 ± 1.0
Control + P50	14.8 ± 0.7	10.5 ± 0.5	25.3 ± 1.2
IM + P0	16.3 ± 0.8	11.2 ± 0.6	27.5 ± 1.4
IM + P50	18.9 ± 0.9	12.8 ± 0.6	31.7 ± 1.5
Glomus clarum + P0	20.2 ± 1.0	13.5 ± 0.7	33.7 ± 1.7
Glomus clarum + P50	23.4 ± 1.2	15.0 ± 0.8	38.4± 2.0

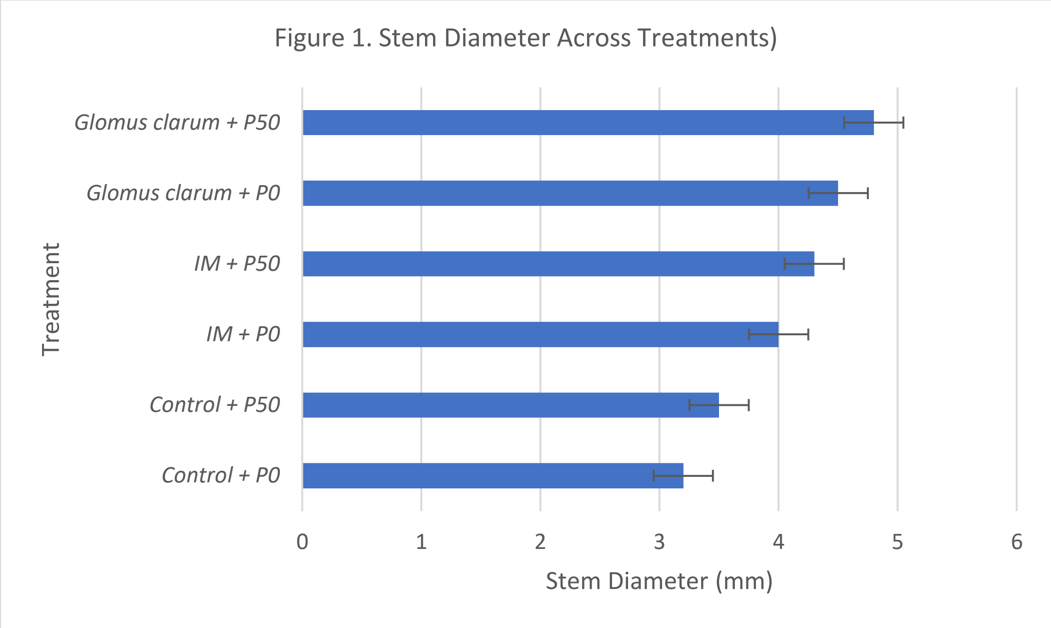
Figure 1. Stem Diameter Across Treatments)
Treatment
Glomus clarum + P50
Glomus clarum + P0
IM + P50
IM + P0
Control + P50
Control + P0
0
1
2
3
4
5
6
Stem Diameter (mm)

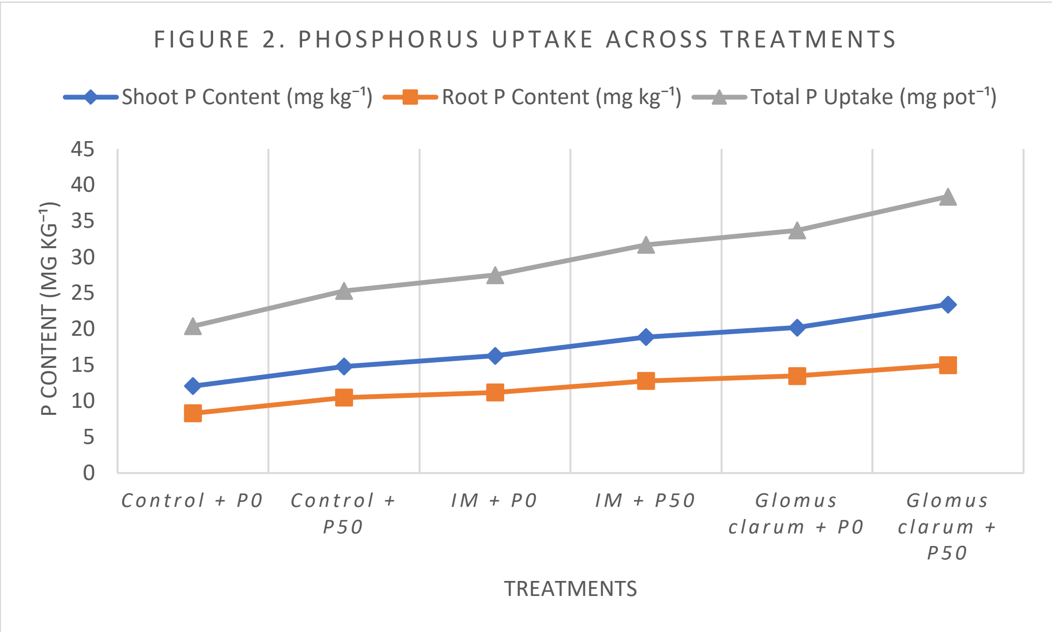
FIGURE 2. PHOSPHORUS UPTAKE ACROSS TREATMENTS
Shoot P Content (mg kg⁻¹)
Root P Content (mg kg⁻¹)
Total P Uptake (mg pot⁻¹)
P CONTENT (MG KG⁻¹)
45
40
35
30
25
20
15
10
5
0
Control + P0
Control + P50
IM + P0
IM + P50
Glomus clarum + P0
Glomus clarum + P50
TREATMENTS