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Research Article

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The Impact of Applying Phosphorus and Mycorrhiza on the Morphological Parameters of Cotton Roots and Shoots, as well as Their Phosphorus Uptake and Dry Matter Yield

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ABSTRACT
This study aimed to investigate the effects of Phosphorus and mycorrhiza application on cotton root and shoot morphological parameters, phosphorus uptake, and dry matter yield. The hypothesis to be tested is that mycorrhiza and P applications increase cotton growth yield and P uptake. The experiment was established under greenhouse conditions in May 2022 and harvested in July 2022, as a pot experiment. In the experiment, two P levels (0 mg P_2O_5 kg^{-1}, and 50 mg P_2O_5 kg^{-1}), three mycorrhizae species (control (without mycorrhiza), indigenous mycorrhiza (IM) and *Rhizophagus clarus* inoculated by May 505-cotton (*Gossypium hirsutum* L.) variety were used with three replications. Before harvesting, plant height and stem diameter were measured. At harvest, shoot and root dry matter weight (DW), P concentration and root morphological characteristics were determined. Mycorrhizal Dependency (MD) was calculated. The results showed that there was no statistically significant difference in terms of plant height, but P and mycorrhiza treatments made a significant difference in terms of stem diameters. In addition, mycorrhiza application made a significant difference in DW. IM application had 45.1 g pot^{-1} and *Rh. Clarus* had 47.38 g pot^{-1} total DW. The results showed that mycorrhiza and P dose applications increased root growth parameters. Mycorrhizae dependency of cotton plant was found to be 8.0 to 12.3%, and MD was lower in P-treated pots (8.1%) compared to non-P-treated pots (11.3%). Results show that mycorrhiza inoculation and 50 mg P_2O_5 kg^{-1} applications seem to be suitable for cotton growth.
Keywords: Phosphorus, Mycorrhizae, Cotton, Dry matter and Root morphology

INTRODUCTION
Cotton is an industrial crop used in many industries such as textile, oil, feed, bio-fuel and paper industries. Cotton increased the diversification of industry and trade, thereby enhancing the country's dollar income, economic contribution, and employment. Over-fertilisation and wrong agricultural practices (such as over-cultivation, irrigation and chemical use) reduce the fertility of soils and negatively affect cotton production. Fertilization of cotton plants is important in terms of fiber and seed quality. P is one of the most consumed elements in terms of quantity for cotton production.

P is involved in many mechanisms, such as pollen tube formation, ATP, ADP and phospholipids. P, which is not a sustainable resource, is assumed to run out in the next 50-150 years (Schnug and Haneklaus, 2016). Plants take P as water-soluble phosphate ions (especially in the form of H_2PO^{4-}) through their roots (Roberts and Johnston, 2015). Plants cannot benefit from insoluble forms of phosphorus. Inadequate P fertilization decreases yields, while excessive P fertilization causes growth reduction and eutrophication. In soils with high pH, the P element forms compounds such as Ca-P (Marschner and Rengel, 2007), while in soils with low pH, it forms compounds such as Fe-P (Yli-Halla, 2016). Plants cannot use these compound forms. For this reason, optimum P fertilization and the use of sustainable resources such as mycorrhizal fungi are of great importance.

Arbuscular Mycorrhiza Fungi (AMF) mutualistically with terrestrial plants (80%) provide water and nutrients (especially P) to the plants, while plants provide photosynthesis products to the mycorrhiza (Smith and Read, 2008). Mutualism between them occurs in this way. It is also known that mycorrhizal fungi increase plant resistance against stress factors (salinity, drought, etc.), disease and pests, and increase flowering and yield (Wahab et al., 2023). In addition, mycorhizal inoculation increases plant root morphological parameters (Chandrasekaran, 2022).

This study aimed to investigate the impact of P fertilization and mycorrhiza application on cotton root and shoot morphological parameters, P uptake and dry matter yield. The hypothesis to be tested is that mycorrhiza and P fertilizer applications increase cotton P uptake, growth, and yield.

MATERIALS AND METHODS

The experiment was established in May 2022 and harvested in July 2022, at the University of Cukurova, Department of Soil Science and Plant Nutrition, under greenhouse conditions, as a pot experiment. Table 1 shows some physico-chemical properties of the Menzilat (*Typic Xerofluvents*) soil series (Staff, 1999) used in the experiment. In the experiment, two P doses (0 mg P_2O_5 kg^{-1}, and 50 mg P_2O_5 kg^{-1}) as P0 and P50, and three mycorrhiza inoculation (control (without mycorrhiza), indigenous mycorrhiza (IM) and *Rhizophagus Clarus* applied with May 505 cotton (*Gossypium hirsutum* L.) variety were used with three replications.

Indigenous Menzilat soil spores were extracted and multiplied via trap culture. Before harvesting, plant height and stem diameter were measured. immediately after harvesting, shoot, root, and total dry matter weight (DW) were obtained. In addition, the P content of the root and shoot parts of the plant was determined by ICP-OES and root morphological characteristics were obtained by WinRhizo software. Mycorrhizal dependency (MD) was calculated by the DW yield of cott (Equal 1) according to (Ortas and Iqbal (2019).

$$M = \frac{DW\ Mycorrhizal\ Plant - DW\ Non\ Mycorrhizal\ Plant}{DW\ Mycorrhizal\ Plant} x100$$

Equal 1.

Table 1. Some physical and chemical properties of the Menzilat soil series (Işik and Ortaş, 2024).

Parameter	Value
pH (1:2.5)	7.42
EC (mmhos cm^{-1})	0.13
Organic Matter (%)	1.15
P_2O_5 (kg da^{-1})	7.00
$CaCO_3$ (%)	35.21
Sand (%)	28.00
Silt (%)	33.25
Clay (%)	38.75
Texture Classification	CL (Clay Loam)

The experiment was designed as a complete randomized design with three replicates for each control, phosphorus and AM treatment. All data were analyzed by using ANOVA, and LSD's multiple range test was used to distinguish the main effects of the various treatments at $p \leq 0.05$. Moreover, the JMP8 statistical program.

RESULTS AND DISCUSSION

Dry Weight

The effects of P and mycorrhizal inoculation on cotton's different parts (root and shoot) and total dry weight (DW) yield were determined. Table 2 shows that P application and mycorrhizal inoculation made a statistically significant difference in root, shoot and total DW. Mycorrhizal inoculation is expected to increase the DW yield of cotton roots and shoots. Previously, Ortas and Iqbal (2019) showed that mycorrhizal inoculation increased cotton's root and shoot dry matter yield. This may be because mycorrhizae provide sufficient nutrients (especially P) and water to the plants. Also, under field conditions, Ortas (2012) reported that cotton plants respond significantly to mycorrhizal inoculation.

P plays an essential role in cotton by enhancing reproductive growth and yield (Iqbal et al., 2020). In our study, P fertilization was expected to increase cotton dry matter yield. There are many studies in the literature that P application increases cotton yield and growth (Ahmad et al., 2009; Iqbal et al., 2020; Saleem et al., 2010).

The fact that mycorrhiza and P co-applications did not make a significant difference in dry matter weight may be due to the fact that the treatments increased plant dry matter yield. This situation supports our hypothesis.

Table 2. The effects of P and mycorrhizal inoculation on cotton in different parts of DW

P Doses	Mycorrhiza	Root	Shoot	Total
		(g pot^{-1})		
P0	Control	4.1±0.8c	37.6±0.8	41.6±1.4
	IM	5.9±0.2ba	39.4±0.7	45.3±0.6
	Rh. Clarus	5.6±0.0b	41.0±0.5	46.6±0.5
Average	*P0*	5.2A	39.3	44.5
P50	Control	5.7±0.2ba	37.8±3.0	42.2±1.6
	IM	5.8±0.6ba	39.1±0.3	44.8±0.5
	Rh. Clarus	6.5±0.5a	40.8±1.7	48.2±0.3
Average	*P50*	6.0B	39.2	45.1
Average	*Control*	4.9B	37.7B	41.9C
	IM	5.8A	39.3BA	45.1B
	Rh. Clarus	6.0A	40.9A	47.4A
	P	**	NS	NS
	M	**	*	***
	P*M	*	NS	NS

The mean of three samples with ± standard error. NS is not significant. *, ** and *** show significant level at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$.

Phosphorus Concentration and Uptake

Phosphorus and mycorrhiza treatments did not make a statistically significant difference in the %P concentration of cotton root parts (Table 3). As shoot parts, there are statistical differences just for P application, while there are no statistically significant differences for mycorrhiza inoculation and P*M interactions. P and mycorrhiza treatments did not make a statistically significant difference in P uptake of cotton shoots and total parts. Moreover, as root parts, there are no statistically significant differences in mycorrhizal inoculation, P application, and M*P interaction. When

root P concentration was examined, it was observed that P concentration is very low, especially at the P0 dose. This may be due to the possibility that no matter how much we clean the roots, soil particles may remain on the root surfaces. In the shoot part of cotton, both statistically and on average, P and mycorrhiza treatments increased cotton %P concentration. Also, there is no difference between P50 x IM and P0 x *Rh. Clarus* applications. As seen in Table 4, in terms of plant P uptake (g pot^{-1}), P, M and P*M interactions did not make a significant difference in the shoot and total cotton parts. Only the application of P on the root made a significant difference. P50 dose application provided the highest P uptake. Our study shows that P application increases plant P uptake. And it was expected. For example, Wang et al. (2009) in their study, show that P application rise cotton P uptake. Mycorrhizal fungi association is most beneficial for crops grown by plants through several mechanisms, like organic acid production and the mycorrhizal hyphae pathway (Elbon and Whalen, 2015). When plant P uptake (nutrition) increases, biomass and yield through photosynthesis will also increase (Veneklaas et al., 2012)



Figure 1. Effect of different mycorrhiza applications (left side), and phosphorus application (right side) on cotton.

Table 3. The effects of P and mycorrhizal inoculation on cotton different parts P concentration

P Doses	Mycorrhiza	Root P (%)	Shoot P
P0	Control	0.006±0.002	0.102±0.009
	IM	0.007±0.001	0.108±0.004
	Rh. Clarus	0.009±0.000	0.112±0.014
Average	*P0*	0.007	0.108
P50	Control	0.013±0.003	0.102±0.028
	IM	0.011±0.002	0.112±0.004
	Rh. Clarus	0.016±0.006	0.127±0.010
Average	*P50*	0.013	0.114
Average	*Control*	0.009	0.102
	IM	0.009	0.110
	Rh. Clarus	0.012	0.120
	P	NS	NS
	M	NS	NS
	P*M	NS	NS

The mean of three samples with ± standard error. NS is not significant. *, ** and *** show significant level at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001.

Shoot Morphologic Parameters

Table 5 shows the impact of P and M application on cotton shoot length and stem diameter. P and M treatments did not make a statistically significant ($p>0.05$) difference in shoot length but increased shoot diameter. Since P application increases plant growth and yield, it is expected to increase plant height. For example, Iqbal et al. (2019) reported that increasing doses of P application also increased cotton shoot length. There are so many studies about mycorrhiza increasing cotton plant growth like length and diameter (Price et al., 1989; Rich and Bird, 1974; Wang et al., 2021). Mycorrhizal fungi are known to provide water and nutrients to the plant. This increased cotton growth (like shoot diameter and length). There is no significant difference in shoot length and diameter in terms of P*M interaction. This supports our hypothesis that there is no significant difference between P and M practices**.**

Root Morphologic Parameters

The effect of P and M treatments on cotton root morphological traits is shown in Table 6. There is no significant difference between root morphological characteristics in terms of P, M and P*M interaction. Table 6 shows that P and M applications increased cotton root morphological characteristics. In particular, mycorrhiza-treated plots had higher root length, surface area, and volume compared to non-mycorrhiza-treated plots. Mycorrhizal inoculation significantly increased root length between 18.8% - 25.5%. As the root morphological characteristics increase, roots get more opportunities to contact with soil and increase nutrient

absorption. Therefore, root morphological characteristics are of great importance.

P fertilization affects plant root morphological characteristics. P application is expected to increase plant root morphological characteristics. Öztürk et al. (2019) previously reported that increasing doses of P application increased maize root morphological characteristics under long-term experiment conditions. This study supports our research findings.

Mycorrhizal fungi affect root morphological characteristics (Liu et al., 2016). Mycorrhiza increases root morphological parameters like length and diameter (Chandrasekaran, 2022; Hetrick, 1991). For example, Soylu et al. (2023) Their study shows that M application increases plant root length and surface area. Tian et al. (2024) reported that mycorrhizal inoculation increased root surface area. These studies support our research findings.

Table 4. The effects of P and mycorrhizal inoculation on cotton plants' different parts P (g pot^{-1}) uptake.

P Doses	Mycorrhiza	Root (g pot^{-1})		Shoot		Total	
P0	Control	0.0003	±0.000	0.038	±0.003	0.039	±0.003
	IM	0.0004	±0.000	0.043	±0.002	0.043	±0.002
	Rh. Clarus	0.0005	±0.000	0.046	±0.006	0.047	±0.006
	P0	0.0004B		0.042		0.043	
P50	Control	0.0007	±0.000	0.039	±0.014	0.040	±0.014
	IM	0.0006	±0.000	0.044	±0.001	0.045	±0.001
	Rh. Clarus	0.0010	±0.000	0.052	±0.004	0.053	±0.003
	P50	0.0008A		0.045		0.046	
	Control	0.0005		0.039		0.039	
	IM	0.0005		0.043		0.044	
	Rh. Clarus	0.0008		0.049		0.050	
	P	**		NS		NS	
	M	NS		NS		NS	
	P*M	NS		NS		NS	

The mean of three samples with ± standard error. NS is not significant. *, ** and *** show significant level at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001.

Table 5. The effects of P and mycorrhizal inoculation on cotton shoot length and diameter.

P Doses	Mycorrhiza	Shoot Length (cm)	Shoot Diameter (mm)
P0	Control	82.0±1.0	5.9±0.6
	IM	84.0±7.9	6.5±0.2
	Rh. Clarus	83.5±4.5	6.3±0.2
Average	*P0*	83.2	6.2B
P50	Control	80.0±4.0	6.5±0.1
	IM	84.0±7.9	6.8±0.2
	Rh. Clarus	90.3±10.7	7.1±0.3
Average	*P50*	84.8	6.8A
	Control	81.0	6.2B
	IM	84.0	6.7A
	Rh. *Clarus*	86.9	6.7A
Average	*P*	NS	**
	M	NS	*
	*P*M*	NS	NS

The mean of three samples with ± standard error. NS is not significant. *, ** and *** show significant level at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001.

From Figure 2, the visual representation helps in quickly identifying which plant traits are closely associated with each other. It is observed that total P and root-specific area show a strong positive correlation with other parameters. Conversely, MD% shows a negative correlation with a few other parameters. In addition, there is also a positive correlation between plant tissue P content and shoot length and diameter.

CONCLUSION

Mycorrhizal dependency of the cotton plant was found to be between 8.0 - 12.3%. In general, mycorrhizal dependency is lower in P-treated pots (8.1%) compared to non-P-treated control pots (11.3%). Dual application of mycorrhiza and 50 mg P_2O_5 kg^{-1} seems to be a suitable combination. The results showed that mycorrhiza and P applications increased root parameters.

CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

Table 6. Effects of P and mycorrhizal inoculation on cotton root morphological parameters

P Doses	Mycorrhiza	Diameter	Length	Surface Area	Volume
		(mm)	(cm pot^{-1})	(cm^2 pot^{-1})	(cm^3 pot^{-1})
P0	Control	0.328±0.065	10189±1492	1058±295	7.3±2.6
	IM	0.343±0.040	12268±4754	1282±394	10.7±2.3
	Rh. Clarus	0.306±0.011	12122±672	1164±30	9.0±0.1
Average	*P0*	0.326	11526	1168	9.0
P50	Control	0.308±0.018	11122±875	1073±26	8.3±0.4
	IM	0.318±0.036	13056±173	1304±130	10.5±2.2
	Rh. Clarus	0.307±0.013	14632±4141	1400±368	10.7±2.6
Average	*P50*	0.311	12937	1259	9.8
Average	*Control*	0.318	10655.6	1065.6	7.8
	IM	0.331	12662.0	1293.1	10.6
	Rh. Clarus	0.306	13376.9	1282.0	9.9
	P	NS	NS	NS	NS
	M	NS	NS	NS	NS
	P*M	NS	NS	NS	NS

The mean of three samples with ± standard error. NS is not significant. *, ** and *** show significant level at P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001.

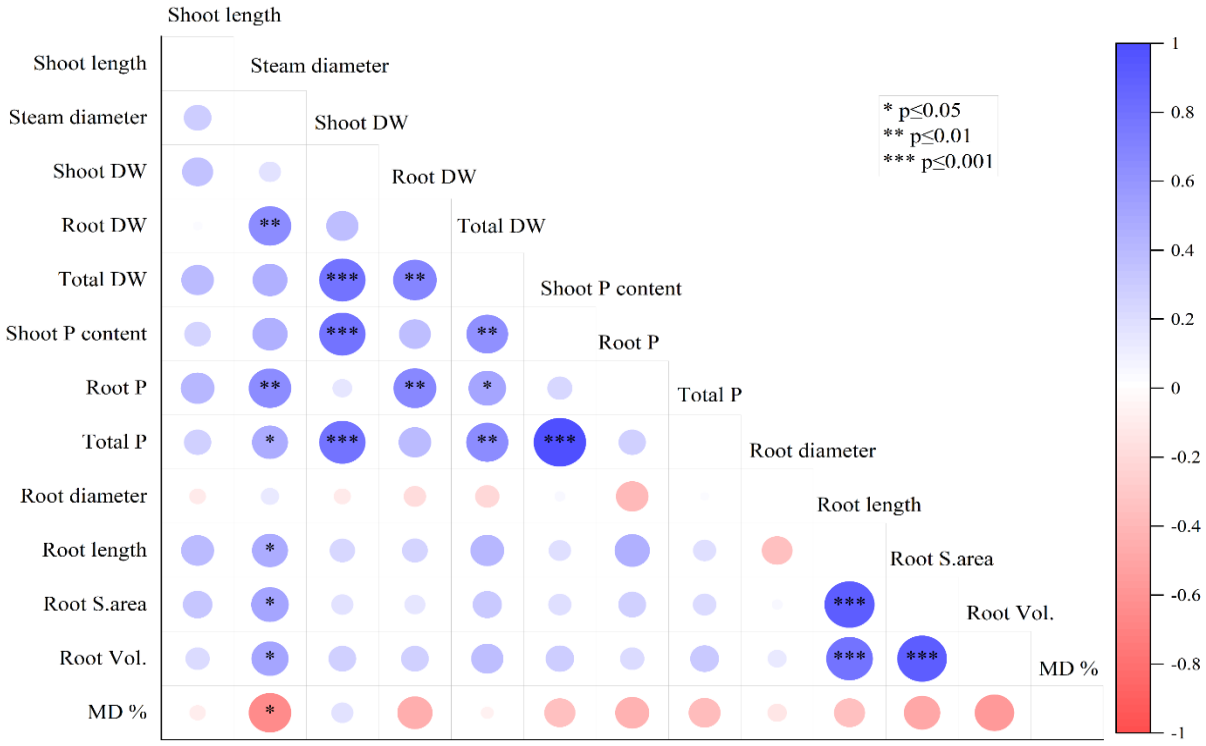


Figure 2. Correlation matrix showing the relationships between the measured plant parameters.

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