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MYCORRHIZA AND *TRICHODERMA* FUNGI ROLE IN IMPROVING SOIL PHYSICAL PROPERTIES PLANTED WITH MAIZE (*ZEA MAYS* L.)

M.Q. HAMID

Department of Soil Science and Water Resources, College of Agriculture, University of Al-Qadisiyah, Al-Diwaniyah, Iraq
Email address: mustafa.qais@qu.edu.iq

SUMMARY

The presented study aimed to investigate the role of an individual and combined bio-inoculation through isolated fungi species (*Trichoderma* and mycorrhiza) in improving the physical properties of sandy soil planted with maize (*Zea mays* L.). The experimental layout was in a randomized complete block design (RCBD) with three replications. The maize seeds sown in plastic pots had soil filling for growing the fungal bio-inoculum on the plant's root hairs. The watering of maize plants used a surface drip irrigation system (DIS). After 60 days of planting, measuring the physical properties of the soil ensued. The combined fungal bio-inoculation treatments (mycorrhiza and *Trichoderma*) had a significant impact on the bulk density and soil porosity, with average values of 1.23 Mg m^{-3} and 53.9%, respectively, compared with the control treatment and their individual treatments. The results showed notable differences among the bio-inoculation fungi treatments, as well as with the control treatment. With *Trichoderma*, mycorrhiza, and their interaction, the average moisture content values were 31.71%, 27.56%, and 47.64%, respectively. For the same three treatments, the average weight diameter values were 0.76, 1.08, and 1.72 mm, and for the saturated hydraulic conductivity of the soil the values were 0.43, 0.48, and 0.16 cm min^{-1}, respectively, compared with the control treatment. Therefore, the fungal bio-inoculation treatments remarkably improved the physical properties of the sandy soil.

Keywords: Maize (*Zea mays* L.), fungal bio-inoculation, *Trichoderma*, mycorrhiza, bulk density, soil porosity, moisture content, weight diameter, saturated hydraulic conductivity

Key findings: The integration of fungal bio-inoculation (mycorrhiza and *Trichoderma*) contributed effectively to improving the physical properties of the sandy soil planted with maize (*Zea mays* L.).

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INTRODUCTION

One of the most blatant problems facing the world at present due to population increase is the shortage of food supplies and its accompanying threat to food security. Most of the cultivated soils suffered with a decline in physical, biochemical, and biological properties. This has also encouraged researchers to address these challenges by developing long-term strategies to improve soil properties with sustainable cropping systems and enhance crops' productivity, including maize (*Zea mays* L.).

Therefore, an earnest need to have safe biological technologies must aim at improving and preserving soil properties and enhancing the quality of crop plants' products (Ijaz *et al.,* 2019; Kenneth *et al.,* 2019). Long-term exploitation of soil leads to a deterioration in soil properties, and this directly affects the health and production of crop plants. Hence, one of the main tasks is to preserve soil properties and enhance its fertility through bio-agricultural techniques (Zhapayev *et al.,* 2023a). The long-term use of mineral fertilizers also considerably affects the physical and dynamic properties of the soil, reducing the soil's supply of nutrients necessary for sustainable plant growth (Bastaubayeva *et al.,* 2023; Makenova *et al.,* 2023).

Biofertilizers are one of the modern technologies in crop sciences. These preparations containing one or more species, and even a combination of beneficial microorganisms added to seeds, soil, and both, enhance the nutrients and improve the soil properties and fertility. This biofertilizer technology involves using useful microorganisms to enrich the physical, chemical, and biological properties of the soil and maintain the balance of nutrients in agricultural soil. Subsequently, it promotes the production and contribution in decreasing the cost of crop production (Verma and Pal, 2020). On the other hand, the bio-inoculation is also vital in binding sandy soil particles, increasing their aggregation due to the adsorption of those cells on the soil surface, and holding sand-attacked particles through protein bridging (Yadav *et al.,* 2011).

Sandy soils have properties that are easily identifiable physically, such as, poor structure and water-holding capacity, high permeability, high sensitivity to pressure, and many other mostly negative effects. However, an analysis showed the prolonged dry season has a significant impact on soil properties, and variations in the soil composition lead to considerable differences in the soil's physical properties (Bruand *et al.,* 2005). The total porosity of the soil is a crucial indicator for studying its structure because it expresses the total area occupied by water and air. Past studies discovered total porosity increases with the addition of bio-inoculations and organic matter (Dorahy *et al.,* 2007).

The surface drip irrigation system is the way to rationalize water use because it uses about 66% less water than continuous surface irrigation and minimizes the risk of harmful plants. Drip irrigation had a watering efficiency of more than 80% compared with other conventional irrigation regimes (Abdul-Razak *et al.,* 2014). Using modern irrigation methods at different levels leads to a significant increase in the amount of moisture needed for better plant growth (Al-Janabi and Al-Rawi, 2018). The presented study aimed to investigate the role of individual and combined bio-inoculation through isolated fungi species (*Trichoderma* and mycorrhiza) in improving the physical properties of sandy soil planted with maize (*Zea mays* L.).

MATERIALS AND METHODS

Using a loamy sand soil sample further attained classification under the order Entisol, sub-order Psamments, the super group, Typical torripsmments, group Calcareous, and the family Mixed Hyperthermia (Al-Atab, 2008).

Taking a soil sample had a depth of 0–30 cm. The soil texture estimation applied the hydrometer method. The particle density measuring used a pycnometer. The bulk density's calculation comprised dividing the mass of dry soil by the total volume of the soil. The total porosity of the soil reached calculation following equation (Black *et al.,*1965) below:

$$P = [1- \frac{pb}{\rho s}] \times 100$$

Where: P = the total porosity of the soil (%), pb = Soil bulk density (Mg m^{-3}), and ρs = Soil Particle density (Mg m^{-3}).

Estimations for calcium, magnesium, chloride, pH meter, and total solid calcium carbonate followed the method according to Jackson (1958). The electrical conductivity measurement proceeded in a soil filter with water in a ratio of 1:1, and the amounts of potassium, sodium, and dissolved sulfate ions also received estimations (Page *et al.,* 1982). Dissolved bicarbonate estimating in soil solution used the technique as mentioned by Richards (1954). The methodology of Papanicolaou (1976) also helped estimate the cation exchange capacity. The results of the physical, chemical, and biological analyses of the soil are available in Table 1.

The experimental treatments included bio-inoculation (mycorrhiza and *Trichoderma*). Using wet sieving and filtering, mycorrhiza spores' isolation from the soil employed the approach by El-Sharkawy *et al.* (2018). The 100 g of soil, taken from around the roots of the plants, sustained air-drying before determining the weight-moisture percentage and then placed in a liter beaker containing 500 ml of distilled water. Shaking well before leaving it for 15–20 minutes. The suspended matter underwent a set of sieves with a diameter of 38, 45, 180, and 250 microns. Then, wash the sieves with a diameter of 38, 45, and 180 microns for residues from the boards, with the impurities collected above each sieve in a clean and sterilized Petri dish. Its examination used an anatomical microscope on a hemocytometer slide to estimate the number of plaques. The soil-added Trichoderma inoculum preparation followed the method described by Sivan *et al.* (1984). All transactions transpired with three replicates, as follows: first - Control treatment (without fungal inoculation), second - Soil inoculation with mycorrhiza, third - Soil inoculation with *Trichoderma*, and fourth - Soil inoculation combined with *Trichoderma* and mycorrhiza.

After being air-dried, soil samples bore autoclave sterilization for one and a half hours (Louis and Lim, 1988). The samples proceeded grinding, passing through a sieve with a diameter of 4 mm, and then placing 8 kg of soil in plastic pots. The addition of bio-inoculation treatments followed, with the plastic pots planted with maize (*Z. mays* L.) seeds. The plants' watering employed the surface drip irrigation (SDI) system using low-salinity water. The surface drip irrigation system consists of a 500-liter water tank, a water pump, an operational pressure gauge, a main line, and three sublines of drippers. As

Table 1. Physical, chemical, and biological properties of the studied soil.

Parameters		Units	Values	Parameters	Units	Values
Particle size distribution				pH (1:1)$_{sups}$	-	8.31
Sand		gm kg^{-1}	840.00	EC (1:1) $_{Ext}$	dS.m^{-1}	1.1
Silt		gm kg^{-1}	110.00	CaCO3	%	1.44
Clay		gm kg^{-1}	50.00	OM	%	0.72
Textural class		-	Loamy sand	Soluble cations		
Bulk density		Mg m^{-3}	1.69	Ca^{++}	meq.L^{-1}	1.13
Particle density		Mg m^{-3}	2.68	Mg^{++}	meq.L^{-1}	1.29
Total porosity		%	36.94	K^{++}	meq.L^{-1}	0.81
Field capacity		%	13.79	Na^{++}	meq.L^{-1}	2.34
Mean weight diameter(MWD)		Mm	0.23	Soluble anions		
Saturation hydraulic conductivity		cm min^{-1}	0.76	$HCO3^{-}$	meq.L^{-1}	3.40
Cation exchange capacity		cmolc/kg^{-1}	1.8	CL^{-}	meq.L^{-1}	4.14
Total fungi		CFU gm^{-1}	$1.1*10^4$	$SO4^{-}$	meq.L^{-1}	2.61
Trichoderma		CFU gm^{-1}	$0.2*10^2$	Available nutrients		
Mycorrhizal		CFU gm^{-1}	$0.5*10^2$	N	Mg kg^{-1}	54.48
Irrigation water properties	pH	-	7.81	P	Mg kg^{-1}	22.98
	EC	dS.m^{-1}	0.92	K	Mg kg^{-1}	240

for the irrigation mechanism, applying drip irrigation ensued when 50% of the water available to the plants reached depletion, as per the following equation (Kovda *et al.,* 1973).

$$d = \left(\frac{Wfc - Ww}{100}\right) \times \rho b \times D$$

Where: d = Depth of water to be added (cm), W_{fc} = Gravimetric humidity at field capacity (%), W_w = Gravimetric humidity before irrigation (%), ρb = Soil bulk density (Mg m^{-3}), and D **=** Depth of effective root zone (cm).

After 60 days of the cultivation, estimation of the physical properties of the soil continued. The wet sieving method used the Yoder device as adopted to mean weight diameter, with estimations according to the method suggested by Kemper and Chepil method (Black *et al.,* 1965). The expression of the results of the mean weight diameter proceeded by applying the following equation.

$$MWD = \sum_{i=1}^{n} xiwi$$

Where: MWD **=** Mean weight diameter (mm), Xi = Diameter rating for any size range of separated assemblies (mm), and Wi = The weight of the remaining aggregates within one size range as a ratio to the total dry weight of the soil sample (g).

The estimation of saturation hydraulic conductivity continued by taking scattered soil samples sifted through a sieve with a diameter of 2.00 mm and saturated with capillary action. The study followed the fixed water column method proposed by Klute (Black *et al.,* 1965), where fixing a column of water above the soil column had the amount of water going through the soil column measured over regular periods until the values became stable with time. The following application of the Darcy law determined if the soil has saturation hydraulic conductivity.

$$K_S = \frac{V}{At} \times \frac{L}{\Delta H}$$

Where: KS = Soil saturation Hydraulic conductivity (cm min^{-1}), V = the volume of water passing through the soil column (cm^3), L = Soil column length (cm), A = the cross-sectional area of the soil column (cm^2), T = Time (minute), and ΔH = L+ Height of the water above the soil column (cm).

Statistical analysis

The experiment had a randomized complete block design (RCBD) with three replications. The data analyses statistically used the SAS program (SAS, 2001). The means' further comparison applied the least significant difference (LSD) test. Further testing the significance of the differences between the coefficients employed the Duncan's Multiple Range Test at the probability level of 0.05 (Duncan, 1955).

RESULTS AND DISCUSSION

Bio-inoculation effect on soil mean weight diameter

For soil mean weight diameter, noteworthy ($P \leq 0.05$) differences emerged due to the combined fungal bio-inoculation (mycorrhiza and *Trichoderma*) against the control and treatments based on the individual application of fungus (Figure 1). The highest value recorded of the mean weight diameter (1.72 mm) appeared with the combined bio-inoculation of both fungi. However, on average, the values for mean weight diameter in the control, mycorrhiza, and *Trichoderma*, amounted to 0.23, 1.08, and 0.76 mm, respectively.

The combined fungal inoculation had a positive impact on the mean weight diameter. The stability of soil aggregates also positively affected on increasing the soil's ability to retain water (Le-Bissonnais, 2023). In addition, the fungi mycorrhiza presence in the environment (through the spread of its hyphae in the ground) enhances the stability of soil aggregates and improves their texture. It improved microbial activity and increased the

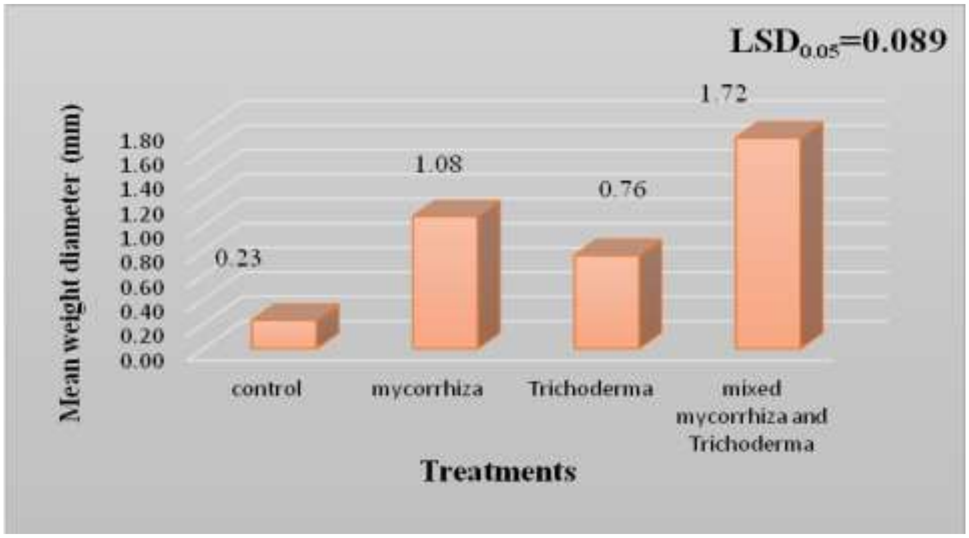

Figure 1. Effect of fungal bio-inoculation on the soil-mean weight diameter (mm).

secretion of sticky glycoprotein compounds (glomalin) from the plant roots. As a result, the binding material for soil particles increased (Fan *et al.,* 2022). Soil micro-biomes drive key functions, determining soil fertility and crop productivity, binding soil particles, and boosting stress tolerance. The micro-biome's intricate linkage with soil structure, caused aggregation and pore connectivity because this structure regulates the flow of water, oxygen, and nutrients through the whole system (Hartmann and Six, 2023).

Crop plants previously inoculated with fungi *Trichoderma* had perfect root systems (Harman, 2000). This root system also enhances the secretions responsible for binding soil particles and forming sand aggregates. The mean weight diameter's significant increase resulted from the adoption of a surface drip irrigation system. This outcome was consistent with past findings and mentioned the surface drip irrigation system (SDI) contributes 13% increase in mean weight diameter (Al-Issawi, 2010). The constant use of water in little amounts and exact quantity required by the plant could be the reason in promoting the growth of aerobic microbes. This irrigation method expels organic matter and gathers them within the root zone due to its leakage prevention mechanism. The presence of aerobic microorganisms aids in improving soil structure and raising the soil mean weight diameter

Bio-inoculation effect on soil moisture content

Significant ($P \leq 0.05$) differences were evident among the combined fungal inoculations, individual application of mycorrhiza and Trichoderma, and the control treatments for the soil moisture content (Figure 2). In combined fungal inoculations, the percentage of moisture content was the highest (47.64%) compared with the lowest value obtained by the control treatment (13.77%). However, in the individual treatments of Trichoderma and mycorrhiza, the moisture percentages were 27.56% and 31.71%, respectively. In soil structure, it seemed a satisfactory value in water stability for plant growth (Kunypiyaeva et al., 2023). Bio-inoculants significantly improve the soil's physical properties, including moisture content, and fungal bio-inoculation plays a key role in improving sandy soil structure, indirectly enhancing the soil's capacity to retain more water (Amooaghaie et al., 2002).

Drip irrigation enhanced the soil moisture content by increasing soil agglomerations and enhancing the soil surface area (Nayyef *et al.,* 2022). This, in turn,

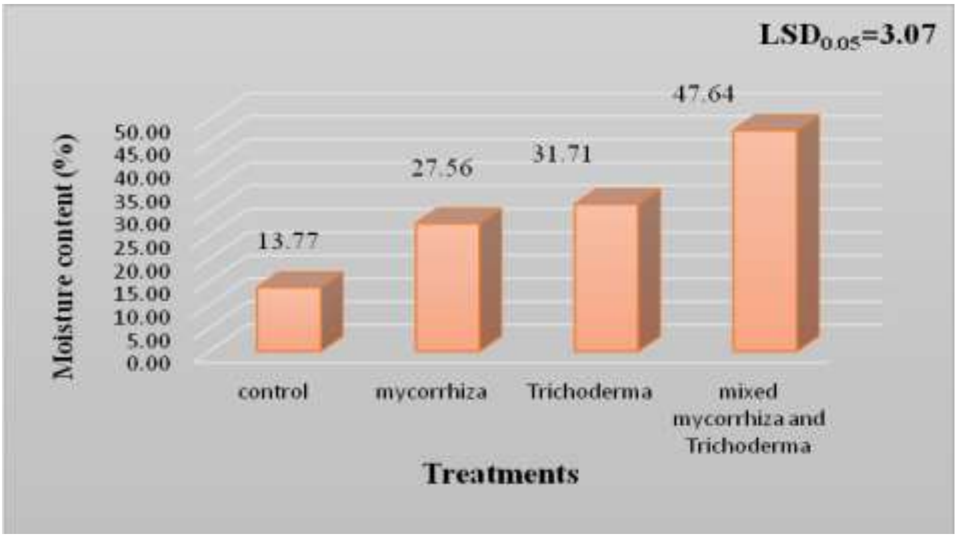

Figure 2. Effect of fungal bio-inoculation on soil moisture content (%).

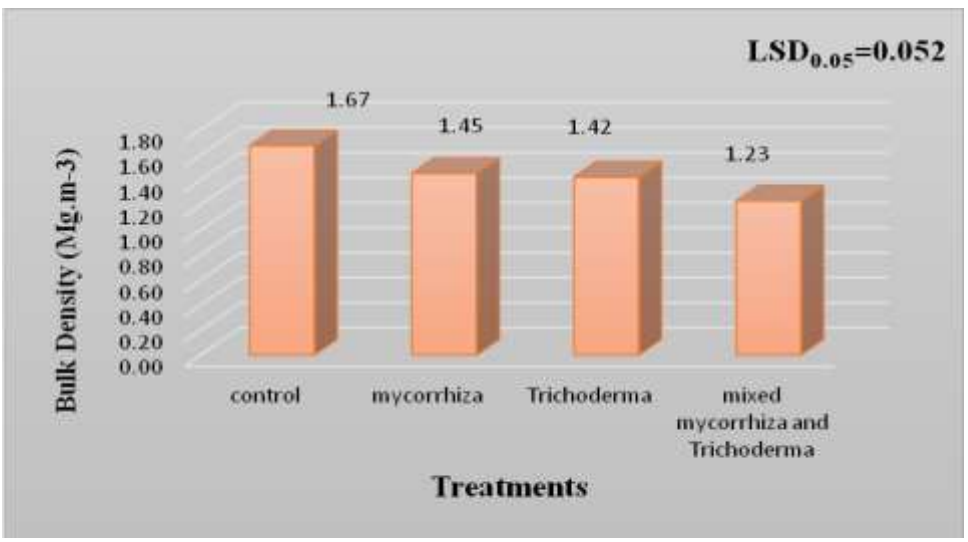

Figure 3. Effect of fungal bio-inoculation on soil bulk density ($Mg.m^{-3}$).

increases the ability of soil particles to retain more water by reducing capillary movement and facilitating plant absorption of water. It also reduced the depletion of irrigation water by the environmental conditions (Verhoef and Egea, 2013).

Salty irrigation water causes a decrease in the values of soil moisture level, the separation of soil grains, and subsequent movement of small particles and their sedimentation in the voids (Ajalloeian *et al.,* 2013). These processes also triggered the formation of cementing material obstructing the water entry into the soil, initiating accumulation of salts in the surface layer, resulting in creating a hard crust on the soil's surface (Dheyab, 2015).

Bio-inoculation effect on soil bulk density

The analysis demonstrated the combined fungal bio-inoculation was significantly ($P \leq$

0.05) superior in reducing the soil bulk density to the ideal value (1.23 Mg m^{-3}), compared with the control treatment (1.67 Mg m^{-3}) (Figure 3). The values of bulk density also decreased with individual application of fungi *Trichoderma* and mycorrhiza (1.45 and 1.42 Mg m^{-3}, respectively). Fungi contribute to raising the total volume of soil, thus, a decrease in bulk density, in addition to their positive role in increasing the stability of soil aggregates (Zhapayev *et al.,* 2023b). The soil structure's fine-tuning and increased soil particle aggregations brought on by the low moisture content, persisting continuously throughout the growing season because of wetting and drying cycles, the bulk density decreased under drip irrigation circumstances (Augeard *et al.,* 2008).

These cycles also encouraged and increased the bulk density gain by an average of 16%. Irrigation with high salinity water leads to a negative effect on density values (Al-Issawi, 2010). Salty water works to crush large soil particles and raise the proportion of smaller ones. In sequence, the sediments in the gaps lead to building semi-compressed layers, causing an upsurge in the bulk density and a decline in the total porosity of the soil (Hassan *et al.,* 2019).

Bio-inoculation effect on total soil porosity

The combined fungal bio-inoculation treatment (mycorrhiza and *Trichoderma*) caused a substantial ($P \leq 0.01$) increase in the total soil porosity compared with the control and individual use of the fungus treatments (Figure 4). The combined fungal inoculation recorded with the highest value for the total soil porosity (54.90%). However, the total porosity values in mycorrhiza, *Trichoderma*, and the control treatments were 45.72%, 48.32% and 37.97%, respectively. This can refer to the positive role of combined fungal bio-inoculation in reducing the bulk density of the soil (Gong *et al.,* 2000). Supporting this hypothesis is an indication that the length of the hyphae correlated to its extension into the soil. The length of a hypha is always many times greater than the root hairs' length. It will extend into the ground and spread with a larger area than the roots, as it can penetrate between the pores of the soil. Transportation within infected is faster because the hyphae are non-septum and can transport more water.

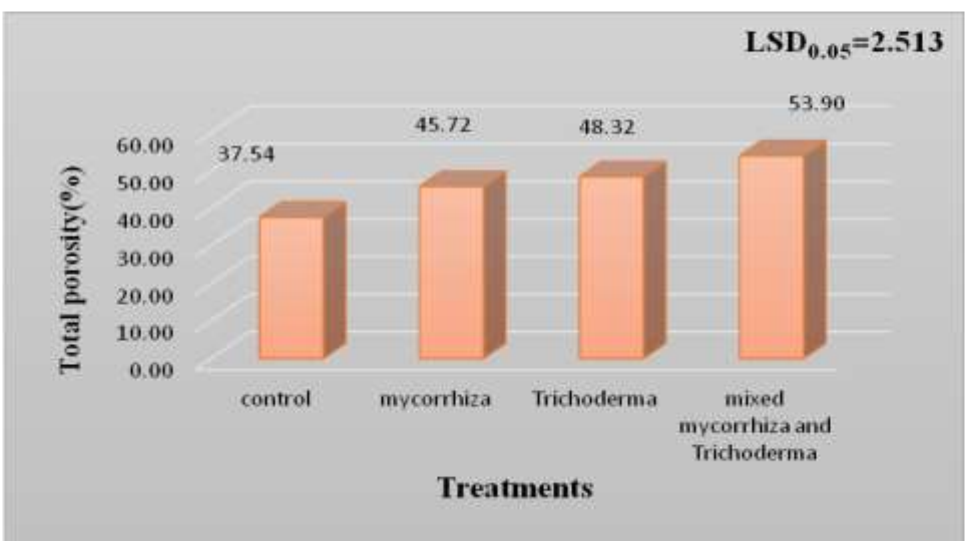

Figure 4. Effect of fungal bio-inoculation on the total soil porosity (%).

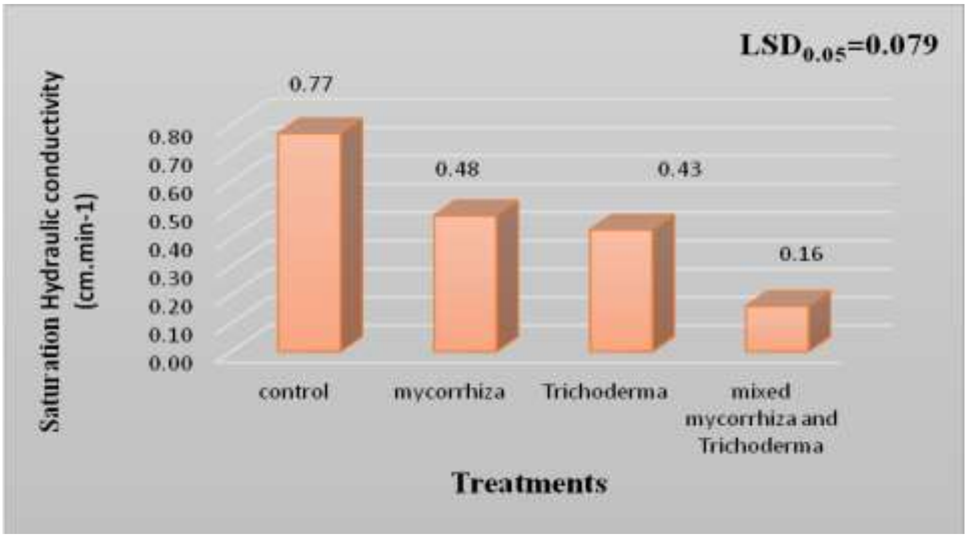

Figure 5. Effect of fungal bio-inoculation on soil-saturation hydraulic conductivity ($cm.min^{-1}$).

Past studies have shown combined bio-inclusion increases the quantity of both living and dead cells, as well as, the adherence of these cells to sand particles (Harit *et al.,* 2021). This adhesion may cause the particles to stick together and decrease the volumes of the interstitial pores. As a result, more holes exist, enhancing the total porosity of the soil in comparison to the uninoculated one. The significant increase in the value of total soil porosity below the SDI was due to the low moisture content sustained throughout the growing season, leading to an improvement in soil structure and an increase in soil particle aggregation (Augeard *et al.,* 2008). Additionally, the occurrence of wetting cycles later also raised the values of the total porosity (Al-Issawi, 2010).

Bio-inoculation effect on soil-saturation hydraulic conductivity

The combined biological inoculation (mycorrhiza and *Trichoderma*) in the sandy soil led to a highly significant decrease in the saturation hydraulic conductivity values (Figure 5). The lowest saturation hydraulic conductivity (10.16 cm min^{-1}) was evident with the combined fungal bio-inoculation compared to the control treatment (10.77 cm min^{-1}). This is due to microorganisms' increase in the rhizosphere and adhesion of cells, causing to make protein bridges among soil particles, increasing water retention within the pores (Büks, 2018). According to Hassan and Abd-Al-Mahdi (2016), many bio-inoculations can glue soil particles together and reduce the diameters of the interstitial pores. As a result, it boosts the soil's ability to retain the largest amount of water within the soil.

Mycorrhiza is vital in improving water relations between soil and plants (Egerton-Warburton *et al.,* 2008). Using water with high salt content has a significant effect on the level of the saturated hydraulic conductivity of the soil. Therefore, the soil structure deteriorates, its porosity decreases, and the saturated hydraulic conductivity of the soil decreases, as well (Ahmad *et al.,* 2016). Irrigating the soil using a surface drip irrigation system boosts the effectiveness of microbes and relatively accelerates decomposition by providing sufficient moisture for the activity of living organisms. Subsequently, it leads to an increase in the decomposition of organic matter in the soil and binds soil fines into aggregates, which improves soil properties, including saturated hydraulic conductivity (Shida and Hamid, 2020).

CONCLUSIONS

The combined bio-inoculation with two types of fungi mycorrhiza and *Trichoderma* under drip irrigation provided encouraging results in improving the physical properties of the sandy soil. Microorganisms have a positive role in incessantly providing the moisture plants needed and maintaining the soil physical properties**.**

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