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THE INTERACTIONS OF PH, ORGANIC MATTER AND SOIL MOISTURE ON ARBUSCULAR MYCORRHIZAL FUNGI (AMF) COLONIZATION IN THE ROOTS OF *Juniperus procera* IN SAUDI ARABIA

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ABSTRACT

The symbiosis between AMF and wild *Juniperus procera* was studied in the soil of Al-Baha, Saudi Arabia. In this study, the effect of soil pH, moisture and organic matter (OM) on the colonization of AMF was investigated. *J. procera* infection by AMF was of the Paris type in which coarse hyphae occupied inter- and intracellular cortical cells. The spores in the soil were identified as *Glomus* sp, *Gigaspora* sp. and *Acaulospora* sp. Percent root colonization with AMF was positively related to pH value ($r^2= 0.81$) and moisture value ($r^2= 0.75$). Nevertheless, AMF colonization in the roots was lower with higher OM content ($r^2= 0.80$). It is important to ensure that soil conditions and properties are maintained in such a way that encourages the growth of AMF and the plant. The use of AMF may be an important consideration in the replanting of this endangered plant species in the future.

Keywords: Arbuscular mycorrhiza fungi; soil pH; OM; soil moisture; *Juniperus procera*

1. INTRODUCTION

Plants living in arid to semiarid ecosystems have developed various ways to combat drought stress (Bray, 1997). One of the most ancient and common plant strategies is the symbiosis of plant roots with AMF so as to improve survival under environmental stress (Brachmann and Parniske, 2006). Although not much is known on the effects of AMF on the water relations and moisture withholding capacity of soils, soil with AMF was found to have considerably more aggregates that are water stable (Augé, 2004). According to Miransari *et al.* (2008), a vast network of hypha and organic matter of elevated quantities are generated by AMF. In Saudi Arabia, the current population of *J. procera* only

represents a small fragment of the woodlands that were once present (Negash, 2002). According to Hajar *et al.* (1991) and Al-Gamdi (2006), juniper forests in the southwest region of Saudi Arabia are exposed to more risks of deterioration because it has low natural regeneration capability and there is a lack of plantation for regeneration. Juniper trees at very low altitudes are also diminishing as a result of soil disturbances and the act of extracting young plants from juniper stands (Hajar *et al.*, 1991).

The hypothesis of this study is AMF infection is abundant in an arid environment of Saudi Arabian soil. There was scarcely any study done on the colonization of AMF in *Juniperous procera* and its relationship to the physical property of soil. This study was undertaken to determine the presence of AMF in the soil and roots and to study the relationship

between AMF in the roots of *J. procera* in the southwest of Saudi Arabia Kingdom and soil pH, OM and moisture which can indirectly affect the growth and development of the trees.

2. MATERIALS AND METHODS

2.1 SOIL AND ROOT HAIRS SAMPLE COLLECTION

Soil and root hairs sample were collected from four areas located in AL-Sarawat (AL-Janabin, Athroub, Shahr, and Hazna) with coordinates ranging from latitude of N 19° 05' to 19° 53' and longitude of E 41° 32' to 41° 43'. At intervals of 20 m, six subareas were selectively chosen. At depths of about 30 cm, five soil samples which also include the roots were randomly collected from each subarea. The roots of *J. procera* trees were also removed of any soil debris and collected into plastic bags to retain its moisture. There were a total of 30 samples per area and the total soil sample for each area was 30 kg. The soil and root hairs were then brought back to the laboratory for further analysis. The soil samples were subjected to pH, OM and moisture analysis, while the roots and soil were examined for AM colonization.

The estimated density of AMF was then calculated based on the equation below:

$$\left(\frac{\text{Number of cells with AMF present}}{\text{Total cells}} \right) \times 100$$

(Brundrett *et al.*, 1996)

2.3 SPORE ISOLATION AND IDENTIFICATION

According to the method proposed by Brundrett *et al.* (1996), AMF spores in 50 g of soil samples were isolated by the wet sieving and decanting method and subsequently subjected to sucrose centrifugation. The supernatant after centrifugation was further sieved using a 50 µm sized sieve, rinsed with water and filtered through a filter paper. Three replicates were carried out for each soil sample.

Isolated spores based on morphological characters were placed on slides and stained with a mixture of Melzer's reagent and

2.2 CLEARING AND STAINING OF ROOT HAIRS

Clearing and staining of root hairs were done according to the methods stated by Brundrett *et al.* (1996) by using hydrochloric acid (HCl), 0.1%, lactoglycerol, Trypan blue stain (0.05%) and potassium hydroxide (KOH) (10%). First, the root hairs were washed with water to remove any soil particles and then treated with 10% KOH (w/v) until the root hairs were sufficiently cleared. Then, the root hairs were placed in an autoclave for about 15-20 minutes at 121°C. The roots were then rinsed with water and further washed with 0.01% HCl. Before the root hairs were transferred into the staining solution (Trypan Blue 0.05%), the roots were again rinsed with water (Brundrett *et al.*, 1996). Acidified root hairs were left overnight in the solution to be stained. Next, the stained roots were removed from the staining solution and destained with lactoglycerol. Microscopic observations were done after the roots were destained. Stained fungal structures can now be easily recognized.

polyvinyl alcohol- lactoglycerol (PVLG) (Morton and Benny, 1990; Koske and Tessier, 1983). The spores were then classified to the genus level by observing the colour, size, wall structure and hyphal attachment (Morton and Benny, 1990) and if possible, the species by using a microscope (CX 41 RF, Olympus Corporation, Philippines) and images were captured with a digital camera (Exwave HAD, Sony, Japan).

2.4 SOIL PH

An amount of 10 ml distilled water was added into a flask containing 10 g of soil. The mixture was stirred well and allowed to stand for 10

minutes. The mixture was then filtered with a filter paper to separate the soil from the water. The pH meter (Mettler Toledo AG) was then placed into the solution to measure the pH level (Conklin, 2005). Before measuring the pH, the ion-sensitive electrode was calibrated using a standard buffer solution of pH 7.

2.5 ORGANIC MATTER CONTENT

A small amount of soil (5 g) was oven-dried at 110°C for 2 hours and then left to cool in a desiccator before weighing. The soil was later placed inside a furnace at 500°C for 3 hours and then left to cool overnight. The incinerated soil was again placed in the desiccator before it was weighed (Wilde *et al.*, 1972). The estimation of OM in the soil sample was obtained by using the formula below:

$$\left(\frac{\text{Dry soil (g)} - \text{incinerated soil (g)}}{\text{Dry soil (g)}} \right) \times 100$$

2.6 SOIL MOISTURE

The moisture content was determined by oven drying 100 g of soil at 105°C for 24 hours. Then, the soil was weighed and placed back into the oven to be dried again. This method was repeated until there was no change in weight loss (Yousef, 1999; Conklin, 2005). The estimation of water content was obtained by using the formula below:

$$\left(\frac{\text{Wet sand (g)} - \text{Dry sand (g)}}{\text{Wet sand (g)}} \right) \times 100$$

2.7 STATISTICAL ANALYSIS

The linear relationship between soil pH, soil OM and soil moisture with the percent of AMF infection was subjected to Pearson's Correlation Coefficient (r^2 value). The Correlation Coefficient provides information on the strength of linear relationship. Values closer to 1 indicate a strong linear relationship between combinations. The relationship between soil pH and roots with the percent of AMF infection were determined by using regression analysis. All analyses were performed using Statistix®

Version 7.0 (Analytical Software, Tallahassee, Florida).

3. RESULT

3.1 MYCORRHIZAL INFECTION IN *J. procera*

The stained roots of *J. procera* were observed under the microscope and found to be heavily infected with AMF about (59-79 %) a lot of endomycorrhizae. A number of endomycorrhizal structures were observed, including hyphal coils that ramify along the root cortex which produces H branches, hyphae with characteristic branching patterns and thin walled intracellular oil-filled vesicles. Thick hyphae were also observed, penetrating the intra- and intercellular region of the cortical tissue. The morphology of the AMF found in the roots of *J. procera* were of the typical Paris type. It was characterized by the presence of intra- and intercellular hyphal branches. Vesicles were present terminally at the tip of the intra cellular hyphal. Arbuscules were also present in the cortical cells (Figure 1a, 2a and 3a).

3.2 AMF SPORES

The spores found in the soil sample belonged to the family of Glomaceae, Gigasporaceae and Acaulosporaceae. The spores were identified as *Glomus* sp., *Gigaspora* sp., and *Acaulospora* sp (Figure 2a, 2b, and 2c). *Glomus* was observed having three layered wall which blends with the wall of the subtending hyphae. *Gigaspora* was characterized by the appearance of the bulbous attachment of the hyphae an the spore. *Acaulospora* was identified by the presence of sporiferous saccule.

3.3 SOIL PH

Figure 3 shows a significant relationship between soil pH and the percent of AMF infection on the roots of *J. procera* ($P < 0.05$). An r^2 value of 0.8909 was recorded, thus showing a strong correlation between the pH value and the AMF infection rate. The pH values in the soil samples were found to be slightly

alkaline ranging from around 7.2 to 7.7. A slight increase in soil pH value showed that the AMF infection rate increased considerably. At pH 7.64, around 79% of AMF was found in the root hairs, while at pH 7.21, AMF infection was only at 59%.

3.4 ORGANIC MATTER

The percent of AMF infection on the roots of *J. procera* were significantly correlated to the percent of soil OM ($P < 0.05$). As the OM in the soil increased, the percent of mycorrhiza decreased (Figure 4). The OM in the soil was relatively low, recording between 3.1-11.4% of the total soil sample. The r^2 value was recorded at 0.7988, showing that the relations between the two variables are strong.

3.5 SOIL MOISTURE

Soil moisture forms a strong and positive relation (Figure 5) with the percent of mycorrhiza infection ($r^2=0.7501$). Soil moisture significantly influenced the percent of mycorrhiza infection ($P<0.05$) in the soil samples. Mycorrhiza infection gradually increased as the moisture level in the soil built up. The highest soil moisture was recorded at around 13.1% and the least was at 4.0%.

4. DISCUSSION

4.1 MYCORRHIZAL INFECTION IN *J. procera*

The mycorrhizas in this study were found to grow and developed well in the root samples of *J. procera*. The association between arbuscular mycorrhizae and their host occurred in many species (e.g. *Octomeles sumatrana* and *Anthocephalus chinensis*) living in different habitats (Chubo *et al.*, 2009). Under harsh conditions such as the deserts, arbuscular-mycorrhizal fungi (AMF) association with plants is a common phenomenon (Stutz *et al.*, 2000). About 95% of 59 plant species in the hot and arid ecosystem in southwest China were associated with AMF (Li and Zhao, 2005). In addition, Uhlmann *et al.* (2006) stated that soil and roots samples collected from three arid sites

in southern Namibia contained AMF, particularly *G. aggregatum*. Ephemerals plants living as desert communities, used for grazing and traditional medicine in Junggar Basin, northwest China were also found to be colonized by AMF and formed typical arbuscules or vesicles structures (Shi *et al.*, 2007).

4.2 AMF SPORES

Glomus is classified under Glomales order, while both Gigaspora and Acaulospora are classified under the order of Diversisporales (Walker and Schüßler, 2004). Glomus is characterized by a three-layered wall that blends with the wall of subtending hyphae (Abbas *et al.*, 2006). Gigaspora lacks intra-radical vesicles and the presence of cells does not vary (de Souza *et al.*, 2005). Gigaspora was characterized by the formation of bulbous structure of the subtending hyphae. Spores from the genus Acaulospora was identified by observing the spores that have separated from the sporiferous saccule as it appears to be stalkless (Abbas *et al.*, 2006).

4.3 SOIL PH

Infection of mycorrhiza in this study was recorded from 59 to 79% which corresponds with an increase in soil pH. Soil pH is one of the criteria that affect the growth and colonization of AMF (Kapoor *et al.*, 2002; Abler, 2004; de Boulois *et al.*, 2008). However, AMF response to soil pH is very much influenced by the type of fungal species. Fungal species can either readily form AMF in soils with low or higher pH (Entry *et al.*, 2002). Kumar *et al.* (2010) found that AM sporulation and the percent of root colonization increased at a higher pH value. It was reported that the number of AM spores and root infection was at its highest at pH 7.2- 7.4 (Selvaraj *et al.*, 2001), while the optimal pH value for mycorrhizal development was reported to vary between pH 6-7 (Sankaranarayanan and Sundarababu, 2001).

4.4 ORGANIC MATTER

In this study, the soil samples contained low amount of OM which is consistent with previous findings. In Saudi Arabia, low amount of OM in the soil was reported by Hashem and Parvez (1994). AMF benefits from the soil surrounding the tree rhizospheres as it contains humified organic matter which mixes with soil minerals (Neelam *et al.*, 2010).

In the Malakand division of Pakistan, a study found that the rate of AMF infection in the roots of wheat and maize crops were correlated to the composition of organic matter. The study found that soils with poor organic matter composition have greater infections with AM (Sharif *et al.*, 2010).

4.5 SOIL MOISTURE

This study found that mycorrhiza infection in the root samples was higher with higher soil moisture. Lack of water is one of the most common problems inhibiting plant growth (Aroca *et al.*, 2008). However, reports have shown that the same physiological processes can be altered through AM symbiosis in addition to helping plants withstand water deficiency (Augé, 2001; Augé, 2004; Aroca *et al.*, 2008).

Under stressful situations such as drought, AMF help plants to grow much better through AMF symbiosis (Porcel *et al.*, 2003). The plant will provide carbon molecules to the AMF and in return the plants will acquire nutrients, particularly phosphorus and water from AMF and this subsequently enhance plant growth (Gosling *et al.*, 2006).

According to Kumar *et al.* (2010), the density of mycorrhiza infection is dependent upon soil moisture. AMF can cause an increase in the absorption of water and nutrients because of the extensive network of hyphae as it increases soil contact with the roots (Miransari *et al.*, 2009).

In arid and semiarid ecosystems, roots of plants usually have a large amount of AM fungal root colonization. This shows that under limited supply of water, AMF symbiosis is of great

importance for plant growth (Tchabi *et al.*, 2008).

Spores germination and development of fungal propagules needs moisture during the early stages of their development despite the fact that AMF helps plant to be more tolerable towards drought (Al-Karaki *et al.*, 2004). Augé (2001) stated that AMF can only provide help in enhancing the tolerability of plants towards drought once the association between AMF and plants has been established.

5. CONCLUSION

In this study, soil physical properties were examined and related to the percent of AMF in the roots of *J. procera*. AMF is known to benefit plants by improving the conditions of the soil. AMF in the roots of *J. procera* was significantly affected by soil pH, soil moisture and organic matter. AMF percent in the roots of *J. procera* increased with an increase of soil pH and moisture level. On the contrary, AMF colonization was high when organic matters were low. Changes in soil pH, moisture and organic matters can influence the abundance of AMF and considerably affect their development.

ACKNOWLEDGEMENT

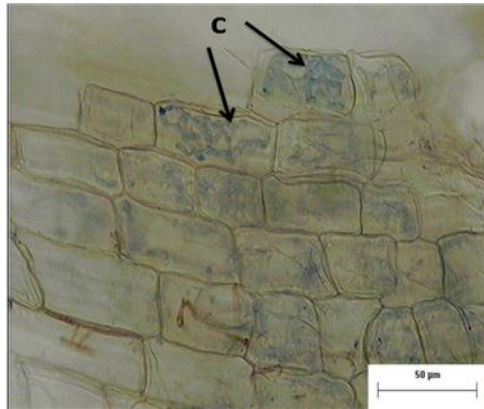
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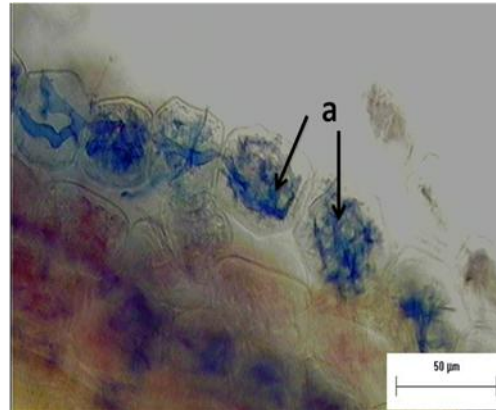
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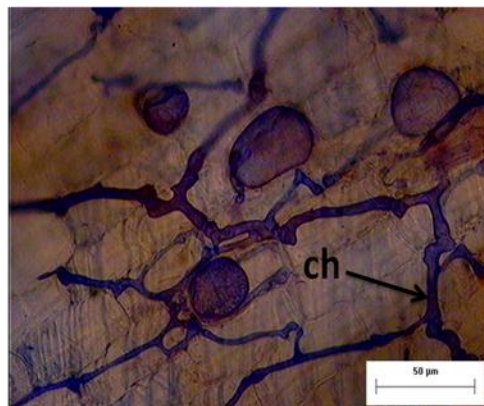
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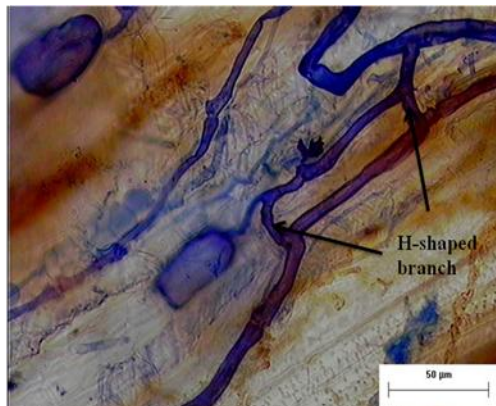
(A)



(B)

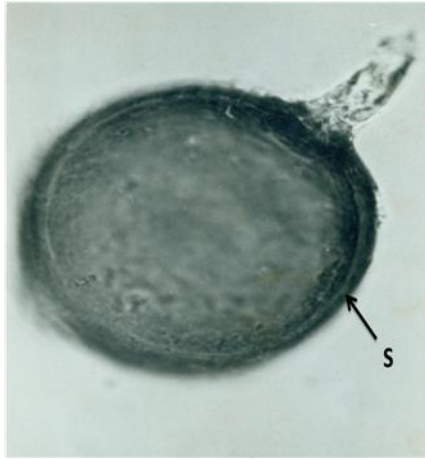


(C)

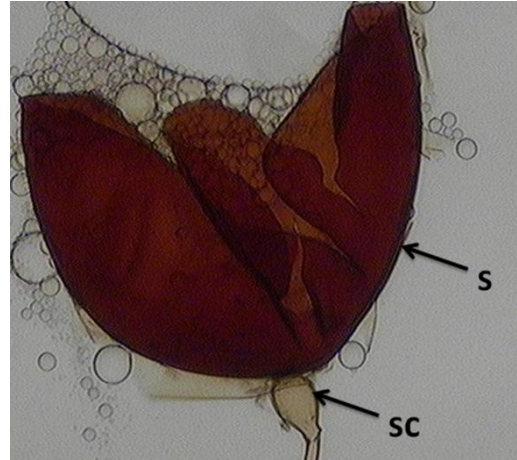


(D)

Fig.1. *J. procera* root colonization by Paris type of AMF showing (A) Coiled hyphae (c) (B) Arbuscules (a) seen in the state of degeneration in the cortical cell of *J. procera*. (C) Morphology of vesicles in the cortical tissues; coarse hyphae (ch) stained blue with trypan blue. (D) H-shaped branch of the internal hyphae of AMF



(A)



(B)



(C)

Fig.2. Spores of (A) *Glomus* sp., (B) *Gigaspora* sp. and (C) *Acaulospora* sp. mounted in PVLG (40x); s= spores; sc= sporogenous cell; ss= sporiferous saccule

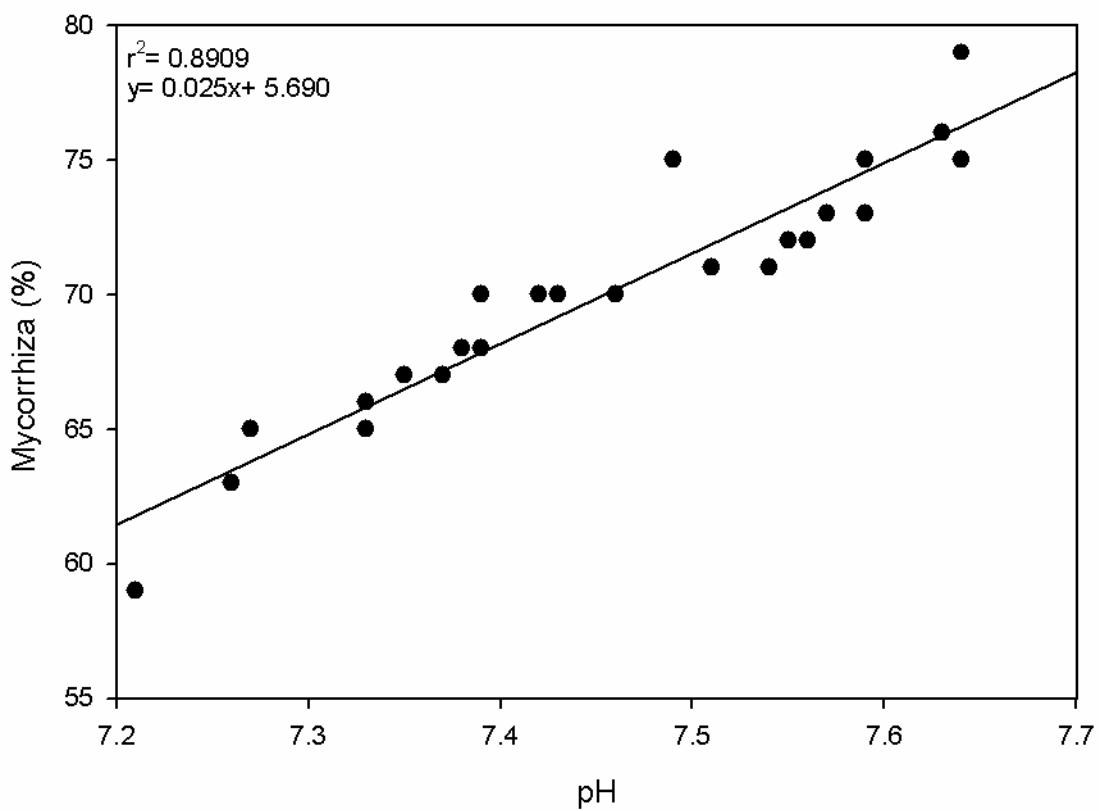


Fig.3. Relationship between soil pH and percent of mycorrhiza infection in the roots of *J. procera*.

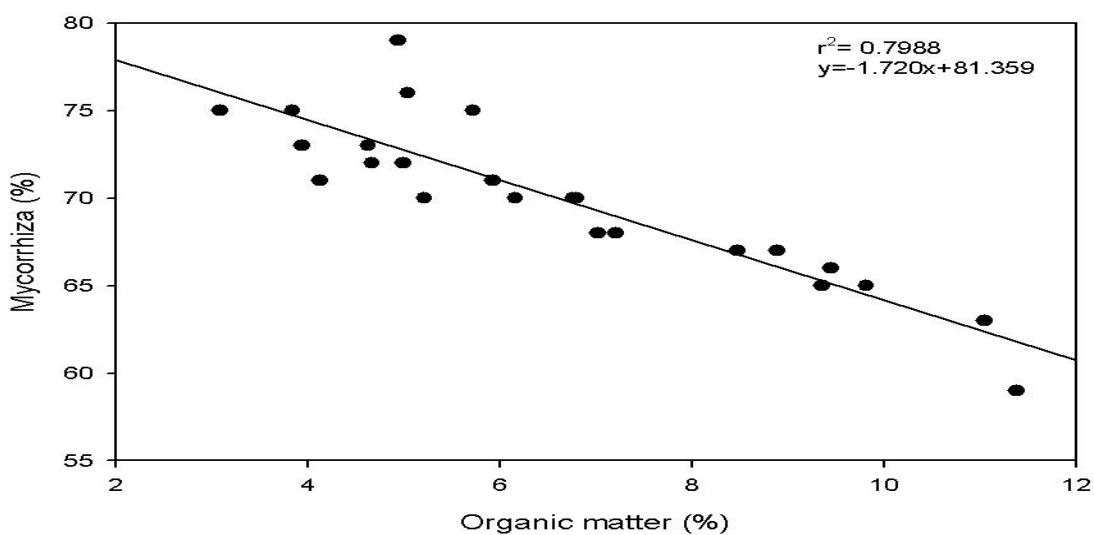


Fig. 4. Relationship between the percent of soil organic matter and percent of mycorrhiza infection in the roots of *J. procera*.

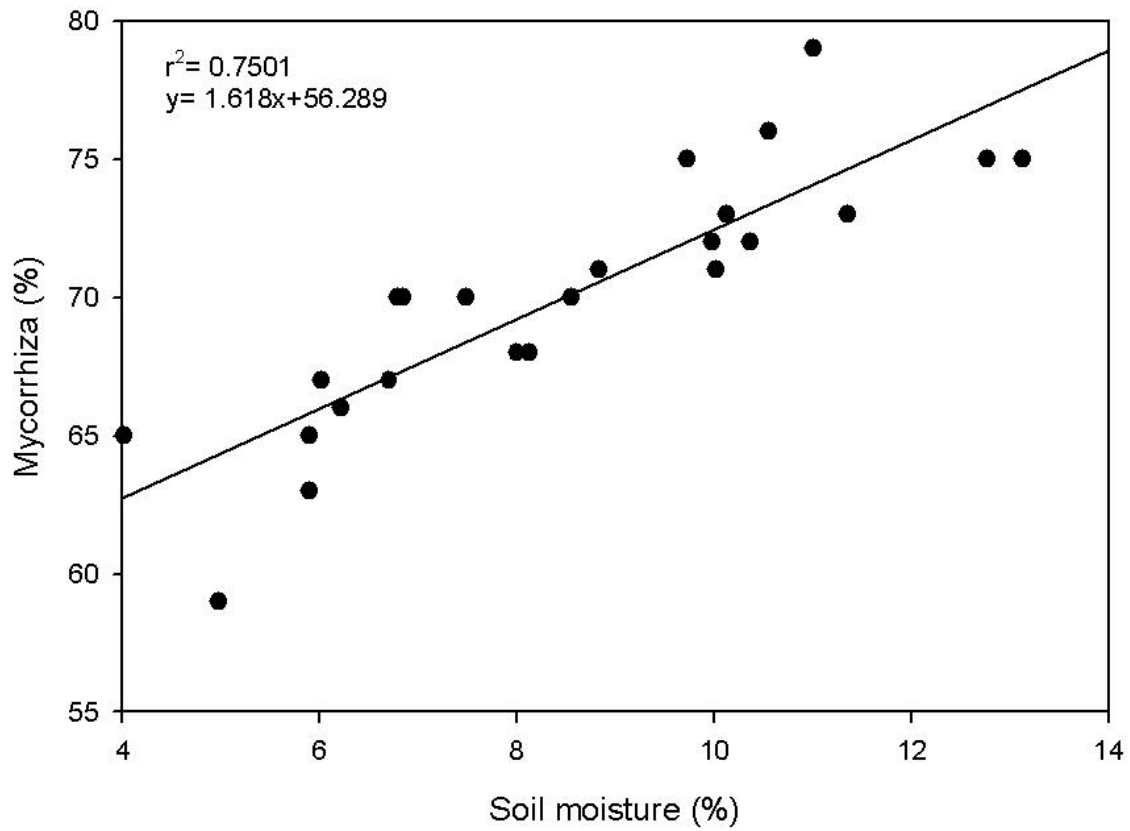


Fig. 5. Relationship between the percent of soil moisture and percent of mycorrhiza infection in the roots of *J. procera*.