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Interaction between soil textural components, flavonoids in the roots and mycorrhizal colonization in *Juniperus procera* in Saudi Arabia

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The degree of infection of *Juniperus procera* roots by arbuscular mycorrhiza fungi (AMF) is highly indicative of the response of plants to environmental stress and is affected by the presence of flavonoids in the roots. However, relatively little is known about the relationship between soil textural components (sand, clay, silt content) and the percentage of AMF in the field soil. The aim of this work was to determine the relationship between soil textural components, content of flavonoids and AMF infection in the roots of *J. procera.* A significant correlation was found between AMF in the roots of *J. procera* and the soil textural components. A positive or negative correlation depended on each composition of soil texture. A negative correlation was found between the percent of AMF in the roots and the amount of coarse sand, fine sand and clay, while a positive correlation existed between the percent of AMF and silt. The degree of colonization with AMF was higher when the quantity of coarse sand was lower (r = -0.83). Similar trend was also observed with fine sand (r = -0.84), and clay (r = -0.83). On the contrary, root colonization with AMF was higher when the quantity of silt was higher (r = 0.92). This study demonstrated that the proportion of silt in the soil and the amount of flavonoid are strongly and positively correlated with AMF infection in the root of *J. procera* in Saudi Arabia.

Key words: Arbuscular mycorrhiza fungi, *Juniperus procera,* flavonoids, soil texture.

INTRODUCTION

Soil texture is known to affect soil microbial population recovery during restoration of grassland (Bach et al., 2010). The high sand content in the grassland also enhanced arbuscular mycorrhiza fungi (AMF) colonization in grassland (Zaller et al., 2011). AMF colonization on roots may influence soil structure by binding soil particles together to form bigger and more stable aggregates (Rilling and Mummey, 2006). Symbiotic associations of plant with arbuscular mycorrhiza conduce to the sustainable maintenance of plant condition and soil fertility (Jeffries et al., 2003) and play an essential part in soil aggregation and structure (Rillig et al., 2002). This is due to the influence of soil structure on several biological, chemical and physical features of soil (Díaz-Zorita et al., 2002; Six et al., 2004).

The extensive network of AMF permits the fungi to augment plant nutrient uptake by enhancing soil structure (Rillig and Mummey, 2006). This is due to the fact that many biological, chemical and physical characteristics of soil are influenced by soil structure (Díaz-Zorita et al., 2002; Six et al., 2004). The formation of soil structure may also be enhanced by the creation of symbiotic associations between roots and AMF (Hallett et al., 2009). Arbuscular mycorrhizas have a part to play in soil structure

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and aggregation (Rillig et al., 2002).

Secondary plant metabolites known as flavonoids are produced by many plants. These metabolites play a crucial part in many relations between plants and microbial infection in the roots (Vierheilig et al., 2002). Flavonoids are made up of a wide group of natural products that have C6-C3-C6 carbon skeleton, or to be more precise, a phenylbenzopyran functional group (Marais et al., 2006). AMF are stimulated as well as inhibited by flavonoids.

The colonization of AMF can be triggered by some flavonoids, which act as chemical signals (Bais et al., 2006). Generally, the alterations that are brought about in the roots due to AMF symbiosis has been studied, but those that occur in the roots are still unknown (Scervino et al., 2009).

In the past, *Juniperus procera* occupied a majority of the forest land in Saudi Arabia. At present, due to persistent human activities, these trees have been reduced to detached patches (IUCN, 2010). The populations of *J. procera* constitute only a small fraction of the woodlands of Asir Highlands of the Kingdom and are showing a global decline (Fisher, 1997). Juniper forests in the southwest region of Saudi Arabia have become prone to destruction because of their low capacity to regenerate and the lack of plantation for regeneration (Al-Gamdi, 2006).

It is important to understand the influence of mineral particles and flavonoids on the AMF partners for future project involving the regeneration for re-plantation of the damaged forest area. This study was carried out to investigate the relationship between AMF on the roots of *J. procera* and soil textural components and quantity of flavonoids in the roots.

MATERIALS AND METHODS

Soil and root hairs sample collection

Soil and root samples were collected from four areas, AL-Janabin, Athroub, Shakran and Hazna. In each area, samples were collected from six subareas with five soil samples randomly selected from each subarea. The samples also included the roots which were dug up at depths of approximately 30 cm. The root hairs and soil samples were taken to the laboratory for further investigations. Soil texture analysis was performed on the soil samples, while the roots were subjected to examination for AMF colonization and quantity of flavonoids.

Soil texture analysis

The soil textural components were determined by using the sieve method as described by Al-Yamani and AL-Desoki (2006). Sieves of different sizes (mesh sizes 0.5, 0.1, 0.05 and 0.005 mm) were arranged by decreasing screen size, with the largest one on the top. The soil particle size were based on the classification by United States Department of Agriculture (USDA) (coarse sand <0.5 mm, fine sand <0.25 mm, silt <0.05 mm and clay <0.002 mm).

Clearing and staining of root hairs

The roots hairs were cleaned with water to remove heavy soil particles and treated with 10% KOH (w/v) until they were cleared of all soil particles. The roots hairs were then placed into an autoclave (121°C) for 15 to 20 min. After this, the roots were rinsed with water and later with 0.01% HCl. The roots were again rinsed with water before they were transferred into Trypan Blue 0.005% (staining solution) and left overnight. The stained root hairs were then removed from the staining solution and destained with lactoglycerol. Examination of stained root hairs for fungal structures was then possible under the microscope where AMF density was determined and calculated (Brundrett et al., 1996).

Isolation and identification of flavonoids

The roots of *J. procera* were collected, dried at 60°C and then grounded into powder form. The powder (300 g) was hen extracted with methanol and concentrated. Next, the solution was placed in a fume hood to allow the solvent (methanol) to evaporate. Hot distilled water was added after 24 h to remove the chlorophyll, lipids and waxes in the extract. A filter paper was then used to filter the aqueous methanolic solution several times and further filtration of the solution was done with ether in a separating funnel. The filtrate was collected and stored for future use so as to obtain two equal volumes. The rest of the solution in the separating funnel was collected and column chromatography (absorbent: polyamide; eluting solvents: ethanol: 70, 80, 90 and 100%) was conducted as stated by Harborne and Mabry (1982). Next, paper chromatography (PC) (filter paper: Whatman no. 1 and 3; developing solvent systems) was carried out to allow the exact determination of the compound of the extraction as well as to quantity the amount of flavonoid detected. Finally, thin layer chromatography (TLC) (dimension: 20 x 20 cm; adsorbent: silica gel plates, aglycones: developing solvent system; BPF: benzene, pyriden, formic acid; spray agents: ammonia) was performed according to the methods of Medic-Saric and Males (1999) on the filtrated solution from the column chromatography and this was done to detect free aglucon. Flavonoids detected from the root were compared and identified using the methods as described by Harborne and Mabry (1982), Markham (1982) and Khogali et al. (2006).

Statistical analysis

Pearson's correlation coefficient (r^2 value) was used to determine the linear relationship between each composition of the soil texture (coarse sand, fine sand, clay and silt) and flavonoids with the percentage of AMF infection in the roots of *J. procera*. Differences in each composition of soil texture (%) were transformed into arcsine values and analysed using one-way ANOVA and means were separated by Tukey's HSD. Statistix® Version 7.0 (Analytical Software, Tallahassee, Florida) was used to perform all statistical analyses.

RESULTS AND DISCUSSION

Soil textural components

A significant relationship was found between the percentage of fine sand, coarse sand, clay and silt, and the percentage of infection by AMF in the roots of *J. procera* ($P < 0.05$). On the other hand, an increase in the amount of silt was strongly associated with a higher percentage of mycorrhizal infection with $r^2 = 0.92$ (Figure 5). In this

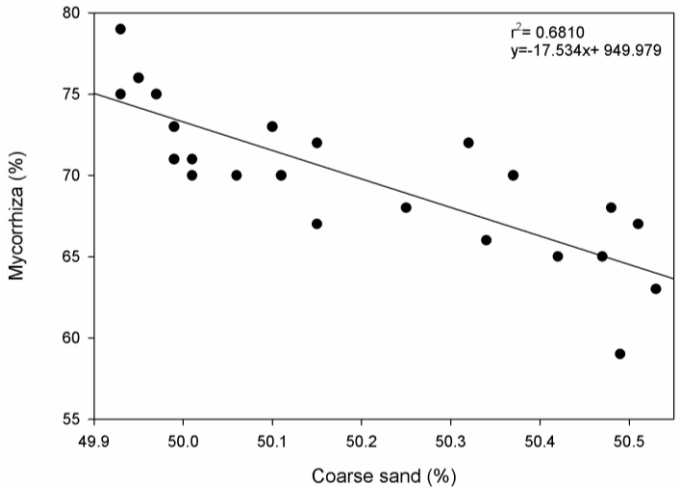

Figure 1. Relationship between the concentrations of coarse sand (%) in the soil and the percentage of mycorrhiza infection in the roots of *J. procera*.

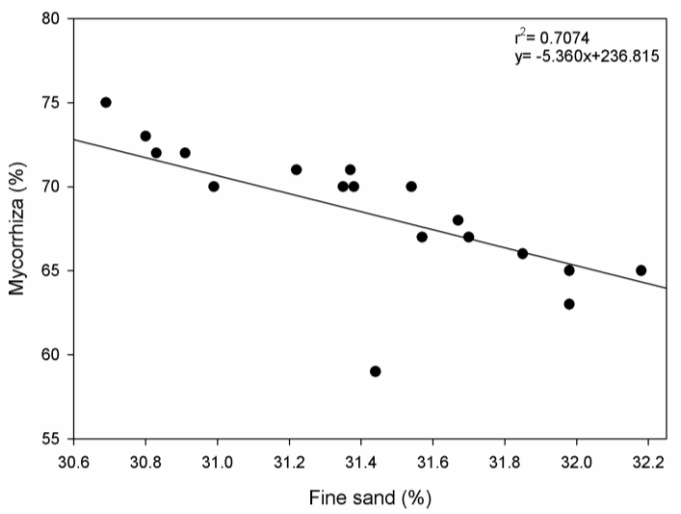

Figure 2. Relationship between the concentrations of fine sand (%) in the shoots and the percentage of mycorrhiza infection in the roots of *J. procera*.

study, there was an inverse relationship between the percentage of mycorrhizal infection and coarse sand, fine sand and clay (Figures 1, 2 and 3). When the amount of coarse sand, fine sand or clay was low, the percentage of mycorrhizae was high and vice versa. This was in accordance with the study by Dakessian et al. (1986), who found that as the coarseness of the soil texture increased, fungal colonisation was negatively affected. The percentage of infection was greater in fine-textured soils than in soil with coarse texture (Djuuna et al., 2010).

Level of AMF colonization increased most rapidly in silty sand soil (Land and Schönbeck, 1991). An r^2 value of –0.83 and -0.84 was recorded for coarse sand and fine sand, respectively, hence showing the strong correlation between coarse sand and fine sand with the rate of AMF infection. About 81% of the soil content in the samples consisted of sand, with coarse sand and fine sand measuring to an average of 50 and 31%, respectively. Results showed that all soil composition (coarse sand, fine sand, clay and silt) was significantly different ($P < 0.05$), with coarse sand measuring significantly the most and clay the least in the total soil (Figure 4).

Although, Saif (1981) stated that the development of AMF is most favourable when the soil is sufficiently aerated, the development of AMF can either be directly or indirectly affected by the presence of sandy soils (Sylvia

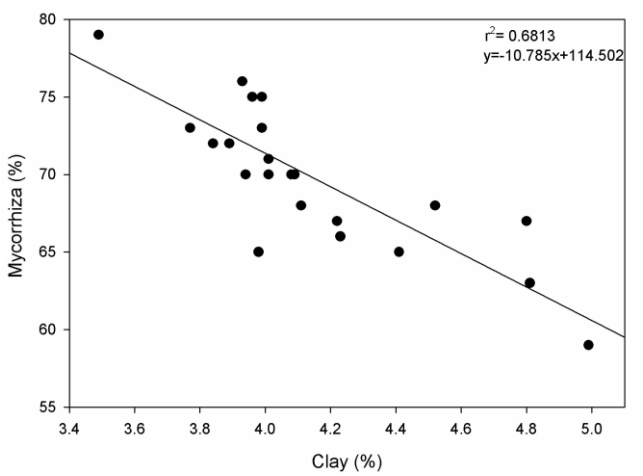

Figure 3. Relationship between the concentrations of clay (%) in the shoots and the percentage of mycorrhiza infection in the roots of *J. procera*.

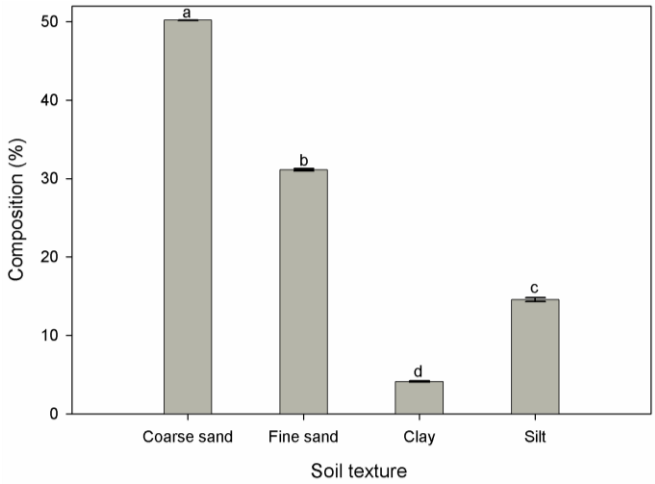

Figure 4. Comparison between different composition (%) of soil texture in the soil (one-way ANOVA; Tukey's HSD, $P < 0.05$).

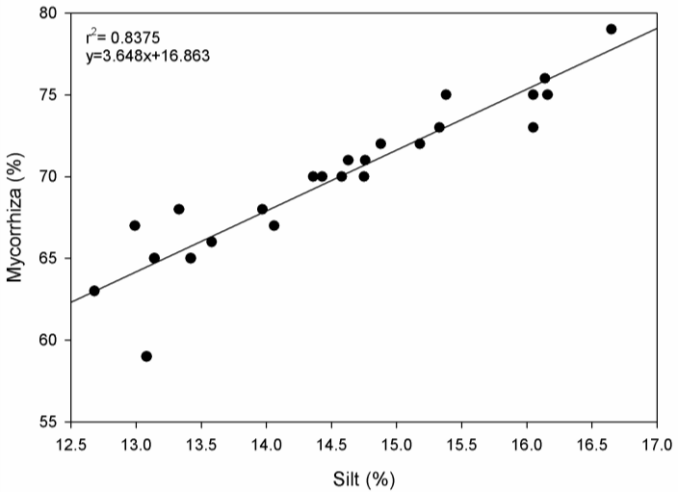

Figure 5. Relationship between the proportion of silt (%) in the soil with the percentage of mycorrhiza infection in the roots of *J. procera*.

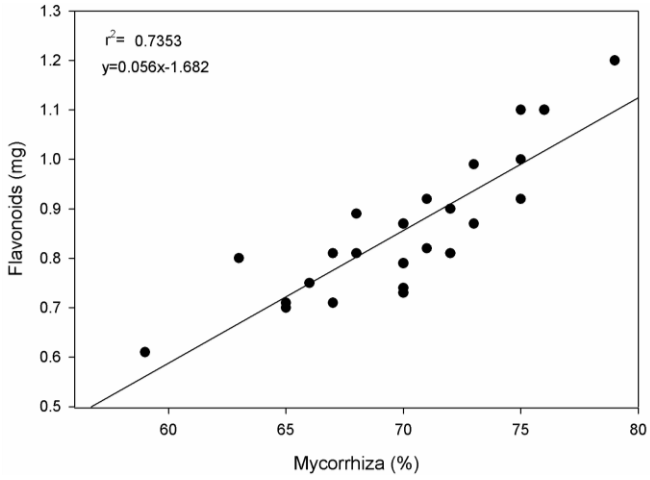

Figure 6. Pearson's correlation between the percent of mycorrhiza infection in the roots of *J. procera* and the presence of flavonoids (mg) in the roots of *J. procera*.

and Williams, 1992). The attributes of sandy soil includes the soil being more porous, warmer but drier and less rich in nutrients when compared to soil which is finer in texture. Soil texture has been reported to affect soil microbial recovery during restoration of grassland (Bach et al., 2010). In some soil texture, the sand content can alter the effects of different mycorrhizal fungi on plant production of biomass (Zaller et al., 2011). Higher sand content also increased percentage AMF colonization.

Moreover, mycorrhizal infection progressively decreased as the level of clay in the soil increased (r^2 = -0.83). Soils with high amounts of clay contain an elevated amount of nutrients as clay can absorb more nutrient ions from the soil solution (Malavolta, 1980). The development of AMF can be limited due to a higher level of nutrients in the soil (Weissenhorn and Leyval, 1996; Eason et al., 1999). However, in this study, the amount of clay in the soil samples was only recorded at an average of 4%. Soils in certain areas such as Michigan in USA are known to have low clay content and this makes the cation exchange capacity of the soil low such that the soil is incapable of retaining nutrients easily (Spurr and Barnes, 1980).

Flavonoids and AMF infection in the roots

Flavonoids content in *J. procera* roots varied from 0.71 to 2.55 mg/kg (Figure 6). It was found that the percentage of AMF colonization in *J. procera* roots significantly affected the amount of flavonoids that is detected in the roots. When the percentage of AMF colonization increased, the quantity of flavonoids in the roots was found to increase (r^2 = 0.8575). Numerous studies found conflicting results on the effect of flavonoids on the development of fungi (Tsai and Phillips, 1991; Bécard et al., 1992; Chabot et al., 1992; Poulin et al., 1997; Vierheilig et al., 1998). It is suggested that flavonoids are common signals for all AMF because of the vast occurrences of AM symbiosis in a majority of plants and the non-specific character of these fungi to host plants (Smith and Read, 1997). Vierheilig and Piché (2002) stated that although there is new information regarding the effects of a variety of flavonoids on AMF growth and induction of flavonoids during colonization of roots by AMF, there are still arguments about the specific role that flavonoids play during the establishment of AM symbiosis.

According to Harrison (1998), exchanges between plant and AMF at a molecular level are present in roots that are colonized by AMF. Root colonization by AMF can be affected by plant flavonoids and these molecules play a significant role in the presymbiotic (germination of spore, length of hypha, branching of hypha and creation of cluster of supplementary cells or secondary spores) and symbiotic stages (creation of entry points and colonization of roots) of the AMF establishment (Morandi, 1996; Vierheilig et al., 1998).

In this study, two types of flavonoids (quercetin 3-rhamnoside and quercetin-3-glucoside) were isolated from the roots of *J. procera.* Certain flavonoids compounds have been recognized to enhance the colonization of roots with AMF (Vierheilig et al., 1998).

Flavonoids of various types and concentrations, and differences in genera and/or species of arbuscular endophytes as well as the difference in the developmental stages of AMF are factors that influence the responses generated by flavonoids towards AMF (Vierheilig et al., 1998). However, the type of flavonoid is not the only factor that determines the effect of flavonoids of AMF; flavonoid concentration also does (Vierheilig et al., 1998; Scervino et al., 2005). Different amounts of flavonoids

present are important as it can have different effects on AMF (Scervino et al., 2009). In various species of fungi such as *Gigaspora* and *Glomus*, quercetin stimulated hyphal growth to a great extent (Bécard et al., 1992; Chabot et al., 1992; Rhlid et al., 1993). A study by Scervino et al. (2005) showed that quercetin stimulated the penetration and root colonization of tomato by *Gigaspora*. The flavonoids present in the roots of *J. procera* may have enhanced the infection of its roots with AMF which subsequently helped it to absorb more nutrients.

Conclusion

This study proved that in sandy soil, higher AMF influence in the roots of *J. procera* was related to the higher content of silt in the soil and higher content of flavonoids in roots.

The presence of AMF is vital in aggregating the soil particles together to form structured soil. Therefore, one of the important reasons for inoculating AMF may have to do with the effect it renders on the soil structure to form aggregates and facilitate water and nutrient holding capacity of the soil. The effects produced by soil textural components have been less studied but our results clearly demonstrated its importance as a regulating factor on mycorrhizal colonization.

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