Journal of Ecology and the Natural Environment Vol. 4(8), pp. 212-218, May 2012
Available online at http://www.academicjournals.org/JENE
DOI: 10.5897/JENE12.023
ISSN 2006 - 9847©2012 Academic Journals

Full Length Research Paper

Relationship between the status of arbuscular mycorrhizal colonization in the roots and heavy metal and flavonoid contents in the leaves of *Juniperus procera*

Amal Ahmed Mohammed AL-Ghamdi[1]*, Hasnah Mohd. Jais[2] and Aisha Khogali[3]

[1]Department of Botany, Environment Program, Faculty of Biological Sciences, King Abdul Aziz University, P. O. Box 35009, Jeddah 21488, Saudi Arabia.
[2]Agrobiology Program, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.
[3]Faculty of Education, Alexandria University, Alexandria, Egypt.

Accepted 30 April, 2012

In the southern region of Saudi Arabia, junipers forest faces the risk of deterioration because of their low regeneration capacity. Arbuscular mycorrhizal fungi (AMF) are known to confer some protection against the various environmental stresses that may affect the regeneration process. This field study was carried out in the junipers forest to evaluate the relationship between the presence of AMF in the roots and the heavy metal and flavonoid contents in the leaves of *Juniperus procera* in its natural ecosystem. Heavy metals present in the leaves of *J. procera* were analyzed, and the flavonoids were extracted, identified and quantified. The colonization of AMF in the roots was found to be significantly correlated with the different concentrations of heavy metals in the leaves. A strong positive correlation (r^2= 0.9172) was found between the percentage of AMF in the roots and the flavonoid concentration in the leaves. Conversely, a negative correlation was found between the concentrations of Cd, Co, Cu, Pb, U and Zn in the leaves and the percentage of AMF in the roots. The relationship between AMF and heavy metal and flavonoid contents in the leaves may provide insight into the dynamics of mycorrhizal symbiosis in the future conservation of *J. procera* in the degraded areas of Saudi Arabian forests.

Key words: Arbuscular Mycorrhizal Fungi, Flavonoids, Heavy Metal, *Juniperus procera*.

INTRODUCTION

Arbuscular mycorrhizal fungus is the most common symbiotic association between fungi and higher plants (Habte, 2000) and about 80% of plant species were reported to be colonized by arbuscular mycorrhizal fungi (Koltai, 2010). In a natural ecosystem, mycorrhizae provide a stable environment for plants to survive by colonizing the root system (Rivera-Becerril et al., 2002). The host plant supplies soluble carbon source to the fungus, while the fungus enhances the ability of plant to absorb water and nutrients from the soil (Entry et al., 2002). The symbiotic relationship between mycorrhizae and plants can also produce new secondary plant metabolites (Venkateswarlu et al., 2008). Several flavonoids such as 2, 4 dihydroxy -4- mehthoxy flavonoid, 5,6,7,8 tetrahydroxydehydroxy-4'-methoxy flavone, qurecetin, acacetin and rhamcetin were found in plant colonized with AMF but not in the uninoculated clover (Ponce et al., 2004).

Flavonoids are phenolic compounds that have both stimulatory and inhibitory effects on AMF. Some flavonoids function as chemical signals that promote the colonization of AMF (Bais et al., 2006), while others have been shown to have a suppressive effect on AMF

*Corresponding author. E-mail: amalalgamdi@gmail.com. Tel: 0060 046534009.

(Wacker et al., 1990). AMF are also known to help the plants to tolerate heavy metals concentrations in the soil (Del Val et al., 1999; Turnau et al., 2005) and contribute to their accumulation in plant tissues (Jamal et al., 2002). It was reported that heavy metal uptake and the root-to-shoot transport via phytoextraction were increased in plants colonized by mycorrhiza (Dodd, 2000). In addition, AMF also play a role in the immobilization of heavy metals within the soil through phytostabilization (Khan, 2005).

In the southwest region of Saudi Arabia, juniper forests face deterioration due to both their low natural regeneration capacity and the lack of replantation effort (Hajar et al., 1991; Al-Gamdi, 2006).

Future conservation and rehabilitation of the forest area should take into account many factors that may be affecting the declining forest. One of the important factors that may be considered is the status of AMF colonization in the roots and its effect on the physiological properties of the plant in the natural forest. In general, the effect of AMF symbiosis on plant physiology has been investigated with regard to the changes that occur in the roots but not in the shoots (Scervino et al., 2009; Al-Ghamdi and Hasnah, 2012). The hypothesis of this study is that in nature the presence of AMF colonization resulted in some effect on the concentration of heavy metals and flavonoids in the leaves of *Juniperus procera*. This study aimed to determine the relationship between AMF in the roots of *J. procera* and the heavy metal concentration and flavonoid content in the leaves.

MATERIALS AND METHODS

Soil and roots sampling

In the present study, the soil, roots and leaves samples was collected between Aprils until October 2009. Sample collection was done in four areas in Al-Sarawat. The four areas are AL-Janabin, Athroub, Shakran and Hazna. The coordinates of the areas ranges from latitude N 19° 05' to 19° 53' and longitude E 41°32' to 41°43. Systematic random sampling of soils, roots and leaves from the experimental sites was adopted due to the impracticality and difficulty of adopting other methods. The systematic sampling involved a system of regularity where a sampling frame was chosen and sampling unit was taken at random. In the present experiment, the sampling frame chosen was the sub areas situated alongside the highways. A sampling unit was the standing plant of *J. procera*. When the chosen unit was without any living plant, the next item was selected. Each sampling unit in a sub areas was separated at intervals of 20 m, thus, six (6) such sampling units were obtained.

From a sampling unit where the standing plant was chosen, a fenced parameter of about one meter radius was made using a plastic rope around the plant. 5 kg of soil was dug about 50 cm deep into the soil. This was done ten times at random. Therefore, 50 kg soil was collected from each sampling unit. The soil was dug at 50 cm deep because of the difficulty in getting the roots sample at shallower depth. In this procedure, collection of soil samples included the roots samples as well.

The 50 kg soil was pooled and homogenized from each sub areas and only 1 kg soil was brought to the lab for further analysis. In total there were 24 (4 areas X 6 subareas), one kg soil samples taken to the lab.

Leave sampling

While sampling the soil and roots, the leaves of *J. procera* was also collected. In each sampling unit, 5 plants of about 1-1.5 m tall were chosen at random. About 3 kg of fresh leaves samples were collected from each plant. Thus 15 kg of fresh leaves sample was collected per sampling unit. The fresh leaves were brought to the lab for further analysis. From 24 sub areas a total of (24 x 15) kg of fresh leaves sample was collected. Prior to the experiment, the fresh leaves sample were air-dried under room temperature for one week. Approximately 300 g of dried leaves sample were used for chromatography study.

Preparation and staining of root

The clearing and staining of roots samples were done using the methods described by Brundrett et al. (1996). The stained roots were then observed under the microscope. Percentage of AMF colonization was then calculated based on the gridline intersection method outline by Brundrett et al. (1996). Stereomicroscope was used to observe the presence of AMF structures such as the vesicles, hyphae and arbuscules that intersected the gridline. The following equation was used for the calculation:

$$\left(\frac{\text{Number of pints with AMF present}}{\text{Total points}}\right) \text{X100}$$

Leaf heavy metal analysis

The analysis of the heavy metals in the leaves was conducted according to the Association of Official Analytical Chemists (AOAC, 1990). After digestion, the samples were cooled, and distilled water was added to a volume of 25 ml. The samples were stored in air-tight bottles for the determination of heavy metals using an Atomic Absorption Flame Emission Spectrophotometer (Model AA670). The experiment was repeated six times for each sample.

Isolation and identification of flavonoids

The collected leaves of *J. procera* were dried for four days at 60°C and then ground into a powder. A 500 g portion of the powder was extracted with methanol and then concentrated; the solutions were left in a fume hood for evaporation. Hot distilled water (100°C) was added to the extract, and the mixture was incubated for 24 h to remove the chlorophyll, lipids and waxes. The aqueous methanolic solution was then filtered, and the solution was further filtered with ether in a separating funnel. To obtain two equal volumes, the filtered solution was collected and kept for later use, and the solution remaining in the separating funnel was collected and subjected to Column Chromatography (CC). The identification of flavonoids was performed accordingly to the methods of Harborne and Marby (1982) and Khogali et al. (2006).

Column chromatography (CC)

Column chromatography was prepared by slowly placing the eluting solvent (70% ethanol) into the column with the use of a glass rod. The mixture was stirred and allowed to settle, excess ethanol was

left to evaporate. The remaining solution was combined with polyamide (adsorbent) and poured into the column chromatography (dimension 6 x 60 cm). This solution was mixed again with the polyamide on the upper part of the column chromatography. Ethanol was released slowly into the column in the order: 70, 80, 90 and 100%. Fractions (50 ml) were then collected into individual beakers (Harborne and Marby, 1982).

Paper chromatography (PC)

A large glass jar was slightly filled with acetic acid: water (15%), and another jar was slightly filled with n-Butanol: acetic acid: water (4:1:5). A drop of each fraction collected from the column chromatography was placed onto filter paper (Whatman No. 1, 20 x 20 cm).

Zorkonium (2 gm) diluted in 100 ml methanol was used to determine the presence of any flavonoids in the fractions. If several spots of flavonoids were detected in a single fraction, this fraction was re-chromatographed using Whatman No. 3 filter paper. The results were then compared to different flavonoids standards (Harborne, 1984), and the flavonoid concentration was determined.

Thin layer chromatography (TLC)

Thin layer chromatography (dimension: 20×20 cm) was performed to detect the presence of free aglucon. The materials used included silica gel plates (adsorbent), aglycones (developing solvent system), benzene, pyriden, formic acid and ammonia (spray agent) (Medic-Saric and Males, 1999).

Statistical analysis

The linear relationship between each heavy metal (Cd, Co, Cr, Cu, Ni, Pb, U and Zn) and the percent of AMF colonization was subjected to Pearson's Correlation Coefficient, which provides information on the strength of the linear relationship: values of r^2 closer to 1 indicate a strong linear relationship between the combinations. The relationship between the heavy metal contents in the soil and roots with the percentage of AMF infection was determined using regression analysis. All of the analyses were performed using Statistix® Version 7.0 (Analytical Software, Tallahassee, Florida).

RESULTS

Heavy metal concentration in the leaves and AMF colonization

The concentration of Cd, Co, Ni, Pb and U in the leaf samples ranged from 0.0 to 0.03, 0.0 to 0.8, 0.0 to 2.3, 0.0 to 0.84 and 0.0 to 0.01 mg/kg, respectively (Figures 1a, b; Figure 2a, b, c). The Cr concentration was found to be in the range of 1.3 to 5.9 mg/kg and the Cu concentration varied from 2.0 to 6.3 mg/kg. Among all of the heavy metals, Zn recorded the highest concentration ranging from 4.3 to 11.9 mg/kg (Figures 1c, d; Figure 2d). In this study, it was found that the concentrations of Cd, Co, Cu, Pb, U and Zn in the leaves of *J. procera* were significantly lower when the percentage of the AMF in the roots was higher ($P < 0.05$). However, the reverse was observed for the correlation between the Cr and Ni concentrations and the AMF colonization (Table 1, Figures 1c and 2a).

Flavonoids in the leaves and AMF colonization of the roots

Four types of flavonoids were isolated from *J. procera* leaves, namely Quercetin 3-rhamnoside, rutin (quercetin-3-rutinoside), quercetin-3-glucoside and quercetin. The flavonoid content in the leaves was found in the range of 1.4 to 2.6 mg/kg (Figure 3). The percent colonization of AMF in the roots was found to correlate with the flavonoids in the leaves of *J. procera* significantly ($P < 0.05$). A strong and positive correlation ($r^2 = 0.91$) was evident from the analysis (Table 2).

DISCUSSION

Arbuscular mycorrhizal fungi (AMF) and heavy metal concentration in the leaves

The percentage of mycorrhizal colonization of the roots was either positively or negatively correlated to the concentration of heavy metals in the leaves of *J. procera*. This trend was similar to the study done on the percentage of mycorrhiza and the concentration of heavy metals in the roots of similar plant (Al-Ghamdi and Hasnah, 2012). In general the amount of heavy metals in the leaves ranged from 2 to 31 folds more than that found in the roots. However, compared to the previous study, the amount of heavy metals in the leaves was up to 2-6000 fold lower than that found in the soil.

In soils that are heavily contaminated with heavy metals, the colonization of AMF in the roots decreases the accumulation of these metals in the shoots, thus protecting the plant against the deleterious effect of heavy metals (Malcova et al., 2003). Therefore, colonization of mycorrhizae in the roots could be of significance in polluted soils that usually have a low nutrient content and low water retention capacity, such as the deserts (Al-Garni, 2006). The decrease in translocation of metals to the shoot has been proposed as an explanation for the observed tolerance of mycorrhizae-colonized plants to heavy metals (Schutzendubel and Polle, 2002). The higher level of heavy metal content in the shoots compared to the roots indicated that translocation of heavy metal occurred from the roots to the shoots and accumulated there (in the shoots). This mechanism is called phytoextraction (Göhre and Paszkowski, 2006).

The field findings in the present experiment showed that uranium was the least heavy metal being accumulated in the shoot that is, 0 to 0.02 mg/kg. Colonization of AM has been shown to sequester

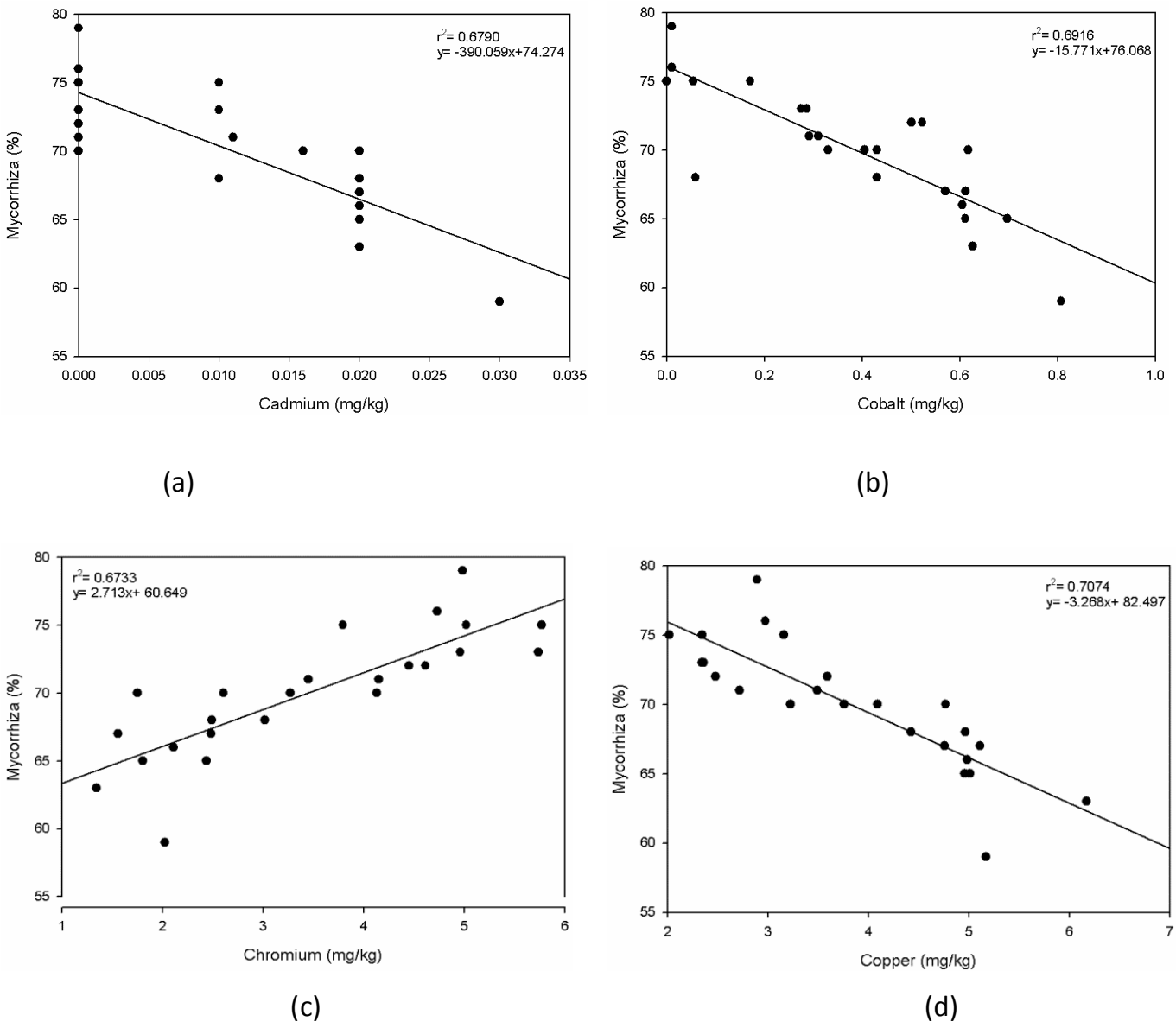

Figure 1. Relationship between the concentration of cadmium (a), cobalt (b), chromium (c) and copper (d) (mg/kg) in the leaves and the percentage of mycorrhiza infection in the roots of *J. procera.*

uranium in the roots and restricts its translocation to the shoots (Chen et al., 2005). It has also been observed that a plant colonized by mycorrhizae has reduced concentration of U in their shoots when grown under increased levels of soil U (Rufyikiri et al., 2004) therefore, protect the plants from the exposure to high degree of U.

When grown in substrates polluted with Cd under various planting conditions, symbiotic associations between AMF and tobacco (variety Wisconsin 38) resulted in reduced level of Cd in the shoots (Janoušková et al., 2005a, b). Growth of *Vitis vinifera* L. (cv. Razaki) was improved by AMF association at high concentrations of Pb and Cd in the substrate (Karagiannidis and Nikolaou 2000).

Depending on the variety of the plant, the fungal associations and the metals involved, several impacts of AMF on the accumulation of metals by plants may be considered as a continuum of responses, from reduced to unaffected to even augmented metal absorption (Malcova et al., 2003); thus, it is difficult to draw any definite conclusions.

In soils containing high levels of Cd, colonization by AMF reduced or had no effect on the foliar levels of the metal (Tonin et al., 2001). Although jackbean roots absorbed Cd and translocated the metal to the leaves, the concentration of the metal was 20 times higher in the roots than in the leaves (De Andrade et al., 2005).

In the case of Zn restriction, the movement of Zn to the

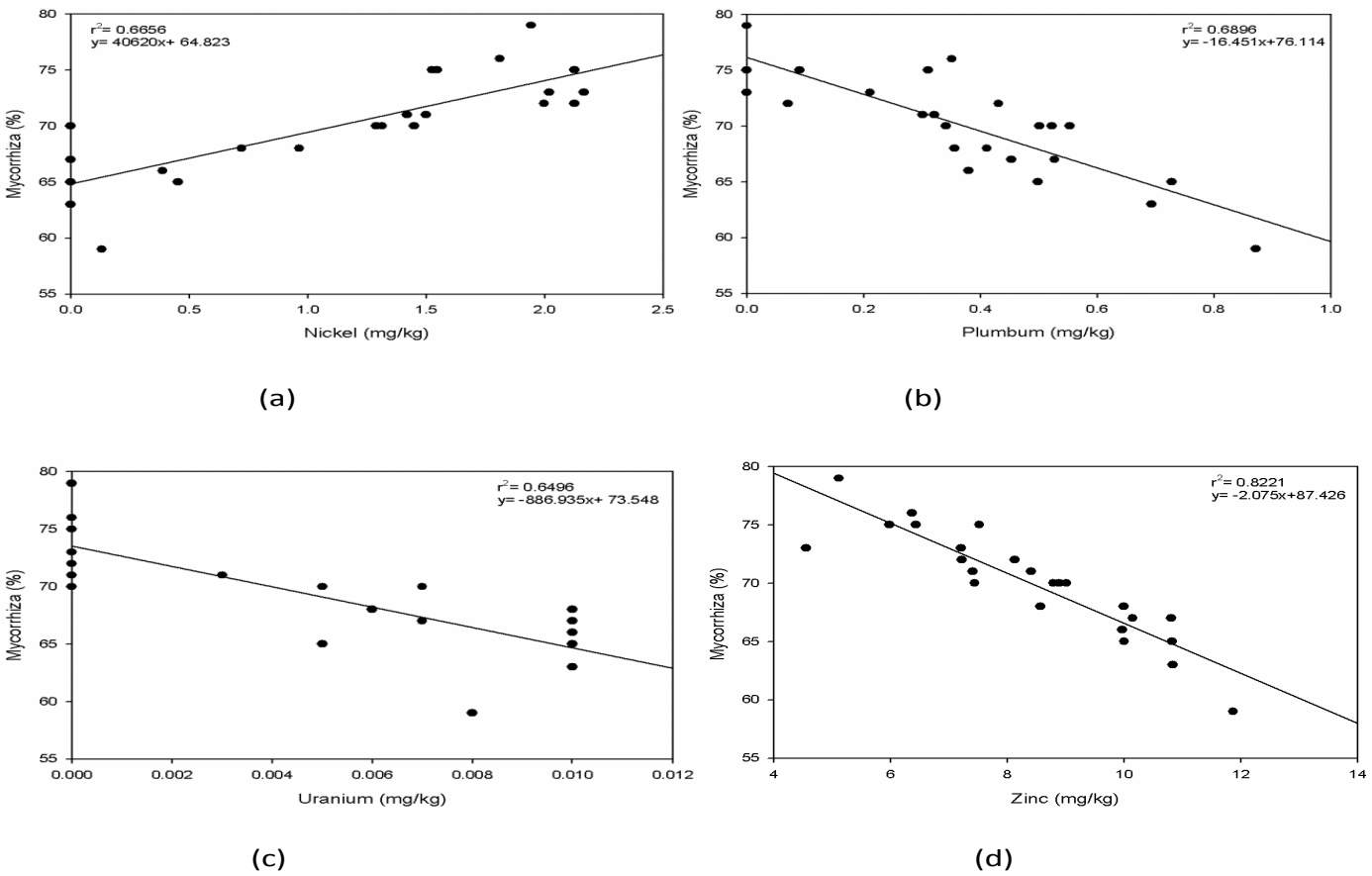

Figure 2. Relationship between the concentration of nickel (a), plumb (b), uranium (c) and zinc (d) (mg/kg) in the leaves and the percentage (%) of mycorrhiza infection in the roots of *J. procera*.

Table 1. Relationship between the concentration of cadmium (a), cobalt (b), chromium (c) and copper (d) (mg/kg) in the leaves and the percentage of mycorrhiza infection in the roots of J. procera.

Heavy metals	r	r^2
Cd	-0.8240*	0.6790
Co	-0.8316*	0.6916
Cr	0.8206	0.6733
Cu	-0.8411*	0.7074
Ni	0.8158	0.6656
Pb	-0.8304*	0.6896
U	-0.8060*	0.6496
Zn	-0.9067*	0.8221

*Correlation is significant at p<0.05.

Table 2. Relationship between the percent of mycorrhiza infection in the roots of J. procera and the presence of flavonoids (mg) in the leaves of J. procera (Pearson correlation).

	r	r^2
Flavonoids	0.9577	0.9173

shoot is augmented by AMF symbiosis (Chen et al. 2003), showing the importance of this symbiosis in the transportation of Zn into the plant (Göhre and Paszkowski, 2006). However, in the presence of high concentrations of Cd and Zn in the soil, colonization by AMF decreased the concentration of Cd and Zn in the shoots of field-grown lettuce and maize (Schüepp et al., 1987). In general, AMF colonization could decrease the concentration of metals in the shoots and, therefore, confer protection against toxic effects to the plants that are grown in soils that are heavily polluted with these metals (Li and Christie, 2001; Malcova et al., 2003).

Flavonoids in the leaves and AMF colonization of roots

In the present work, at least four types of flavonoids were found in *J. procera* leaves. A flavonoid identified as tetrahydroxyflavone, has been isolated from the leaves of *J. procera* growing in Enemas, the southern region of Saudi Arabia (Mujwah et al., 2010) however no AMF study was related in their population. The presence of different amounts of flavonoids is important because

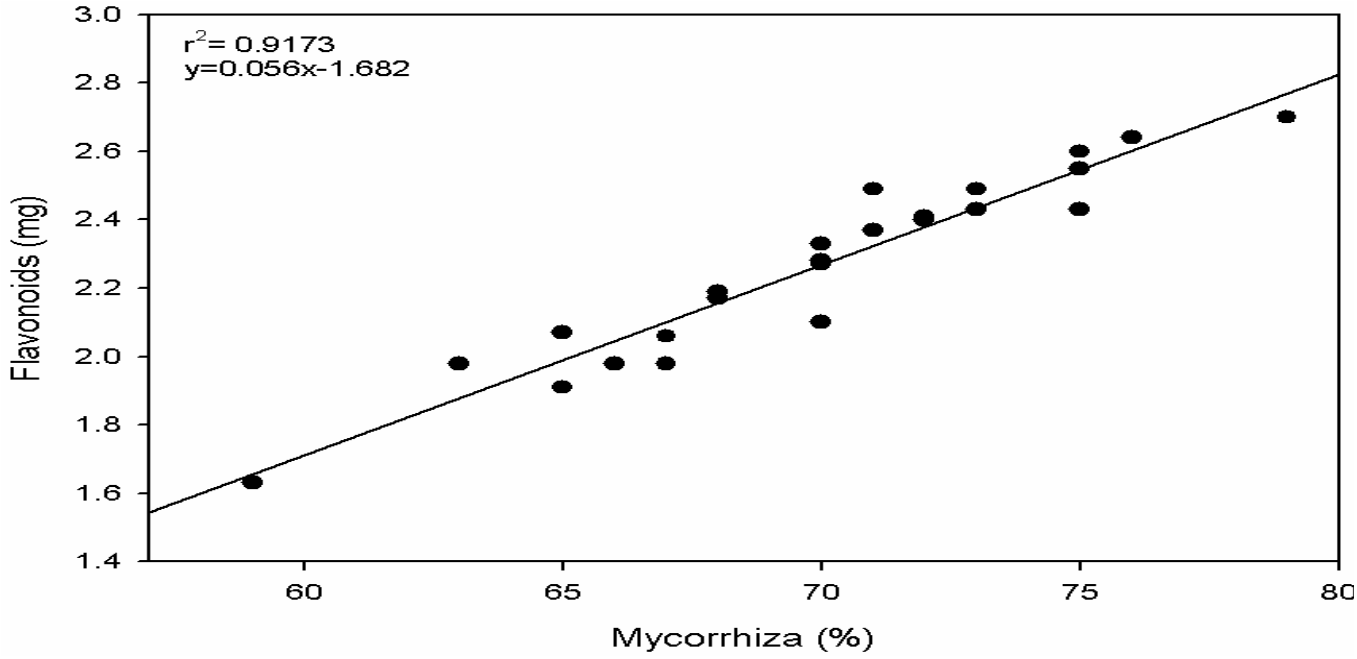

Figure 3. Relationship between the percent of mycorrhiza infection in the roots of *J. procera* and the presence of flavonoids (mg) in the leaves of *J. procera* (Pearson correlation).

these compounds can have different effects on AMF (Scervino et al., 2009). Formononetin, a flavonoid, has been described to augment AMF sporulation and colonization (Juge et al., 2002). In addition, Ponce et al. (2004) reported that the flavonoid pattern in white clover was modified after the colonization by *Glomus intraradices*. However, Scervino et al. (2005) could not establish a distinct relationship between the concentration of the analyzed flavonoids and their influence on AMF colonization.

The strong correlation between AMF percent of colonization and concentration of flavonoid suggest that there is a possibility of some associated functions between the two parameters. Larose et al. (2002) suggested that the flavonoids produced as a result of mycorrhizal colonization could help to increase the resistance level of mycorrhizal plants against soil-borne plant pathogens. The flavonoids level found in the leaves samples of *J. procera* could, at least in some way, protect the plant against certain diseases or infections. Although studies of the colonization by AMF is focused on the roots, the association between AMF and its host plant has been reported to cause physiological and molecular changes in both the roots and shoots (Taylor and Harrier, 2003).

Conclusion

This study showed that in the natural woodland in *J. procera* in Saudi Arabia the roots were strongly associated with the presence of mycorrhizae and the concentration of heavy metals and the amount of flavonoids in the leaves.

ACKNOWLEDGEMENTS

The authors wish to thank King Fahd Medical Research Center, Jeddah, Saudi Arabia, for their assistance. We also thank Universiti Sains Malaysia (Penang, Malaysia) for supporting this study. This work was financially supported by King Abdul Aziz University (Jeddah, Saudi Arabia).

REFERENCES

Al-Gamdi MAA (2006). The possibility of ameliorating the regeneration of Juniper trees (*Juniperus procera* Hochst. ex Endl.) in the natural forests of Saudi Arabia. Riyadh. MSc. Thesis, University of King Saud, Riyadh, King Saudi Arabia.

AL-Ghamdi AAM, Hasnah MJ (2012). Interaction between Arbuscular Mycorrhiza and Heavy Metals in the Rhizosphere and Roots of *Juniperus procera*, Int. J. Agric. Biol., 3: 66-76.

Al-Garni SMS (2006). Increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer *Rhizobium* bacterium, Afr. J. Biotechnol., 5: 133-142.

AOAC (1990). Official methods of analysis. 15th ed. Association of Official Analytical Chemists. AOAC Inc. Arlington, Virginia, USA.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006). The role of root exudates in rhizosphere interactions with plants and other organisms, Annu. Rev. Plant Biol., 57: 233-266.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996). Examining mycorrhizal associations. In: Working with Mycorrhiza in Forestry and Agricultur, M. Brundrett, N. Bougher, B. Dell, T.

Grove, N.Malajczuk, eds., CSIRO Publishing, Canberra, pp.173-215.
Chen B, Zhu Y-G, Zhang X, Jakobsen I (2005). The influence of mycorrhiza on uranium and phosphorus uptake by barley plants from a field-contaminated soil, Environ. Sci. Pollut. Res., 12: 325-331.
Chen BD, Li XL, Tao HQ, Christie P, Wong MH (2003). The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. Chemosphere, 50: 839-846.
De Andrade SAL, Jorge RA, da Silveira APD (2005). Cadmium effect on the association of jackbean (*Canavalia ensiformis*) and arbuscular mycorrhizal fungi, Soil Sci. Plant Nutr., 62: 389-394.
Del Val C, Barea JM, Azcón-Aguilar C (1999). Diversity of arbuscular mycorrhizal fungus population in heavy metal contaminated soil soils, Appl. Environ. Microbiol., 65: 718-723.
Dodd JC (2000). The role of arbuscular mycorrhizal fungi in agro- and natural ecosystems, Outlook. Agr., 29: 55-62.
Entry JA, Rygiewicz PT, Watrud LS, Donnelly PK (2002). Influence of adverse soil conditions on the formation and function of arbuscular mycorrhizas, Adv. Environ. Res., 7: 123-138.
Göhre V, Paszkowski U (2006). Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation, Planta, 223: 1115–1122.
Habte M (2000). Mycorrhizal fungi and plant nutrition. In: Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture, Silva JA, Uchida R (eds), College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa, United states, pp. 127-131.
Hajar AS, Faragalla AA, Al-Ghamdi KM (1991). Impact of biological stress on *Juniperus excelsa* M. Bieb. in south-western Saudi Arabia: insect stress, J. Arid Environ., 21: 327-330.
Harborne JB (1984). Phytochemical methods. 2nd ed., Chapman & Hall, London, p. 275.
Harborne JB, Mabry TJ (1982). The flavonoids: advances research. Chapman & Hall, New York, 1, pp.744.
Janoušková M, Pavlíková D, Macek T, Vosátka M (2005a). Influence of arbuscular mycorrhiza on the growth and cadmium uptake of tobacco with inserted metallothionein gene, Appl. Soil Ecol., 29: 209-214.
Janoušková M, Pavlíková D, Macek T, Vosátka M (2005b). Arbuscular mycorrhiza decreases cadmium phytoextraction by transgenic tobacco with inserted metallothionein, Plant Soil, 272: 29-40.
Jamal A, Ayub N, Usman M, Khan AG (2002). Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soybean and lentil, Int. J. Phytorem., 4: 205-221.
Juge C, Samson J, Bastien C, Vierheilig H, Coughlan A, Piche Y (2002). Breaking dormancy in spores of the arbuscular mycorrhizal fungus *Glomus intraradices*: A critical cold-storage period, Mycorrhiza, 12: 37-42.
Karagiannidis N, Nikolaou N (2000). Influence of arbuscular mycorrhizae on heavy metal (Pb and Cd) uptake, growth, and chemical composition of *Vitis vinifera* L. (cv. Razaki), Am. J. Enol. Viticul., 51: 269-275.
Khan AG (2005). Role of soil microbes in the rhizospheres of plants growing on trace lement contaminated soils in phytoremediation, J. Trace Elem. Med. Biol., 18: 355-364.
Koltai H (2010). Mycorrhiza in floriculture: difficulties and opportunities. Symbiosis, 52: 55-63.
Khogali A, Hajar AS, AL-Ghamdi AAM (2006). Compartive autecological studies on *Ricinus communis* L. (Euphorbiaceae) one of the economical plants growing in Saudi Arabia, Bull. HIPH., 36: 629-644.
Larose G, Chenevert R, Moutoglis P, Gagne S, Piche´ Y, Vierheilig H (2002). Flavonoid levels in roots of *Medicago sativa* are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus, J. Plant Physiol., 159: 1329-1339.
Li XL, Christie P (2001). Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil, Chemosphere, 42: 201-207.
Malcova R, Vosatka M, Gryndler M (2003). Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L. and *Agrostis capillaris* L, Appl. Soil Ecol., 23: 255-267.
Medic-Saric M, Males Z (1999). Selection of an optimal set of solvents in thin layer chromatography of flavonoids and phenolic acids of *Lavandula flos,* Pharmazie, 54: 362-364.
Mujwah AA, Mohammed MA, Ahmed MH (2010). First isolation of a flavonoid from *Juniperus procera* using ethyl acetate extract, Arabian J. Chem., 3: 85-88.
Ponce MA, Scervino JM, Erra-Balsells R, Ocampo JA, Godeas AM (2004). Flavonoids from shoots and roots of *Trifolium repens* (white clover) grown in presence or absence of the arbuscular mycorrhizal fungus *Glomus intraradices*. Phytochemistry, 65: 1925-1930.
Rivera-Becerril F, Calantzis C, Turnau K, Caussanel J-P, Belimov AA, Gianinazzi S, Strasser RJ, Gianinazzi-Pearson V (2002). Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes, J. Exp. Bot., 53: 1177-1185.
Rufyikiri G, Huysmans L, Wannijn J, Van Hees M, Leyval C, Jakobsen I (2004). Arbuscular mycorrhizal fungi can decrease the uptake of uranium by subterranean clover grown at high levels of uranium in soil, Environ. Pollut., 130: 427-436.
Scervino JM, Ponce MA, Erra-Bassells R, Vierheilig H, Ocampo JA, Godeas A (2005). Flavonoids exhibit fungal species and genus specific effects on the presymbiotic growth of *Gigaspora* and *Glomus*, Mycol. Res., 109: 789-794.
Scervino JM, Ponce MA, Monica ID, Vierheilig H, Ocampo JA, Godeas A (2009). Development of arbuscular mycorrhizal fungi in the presence of different patterns of *Trifolium Repens* shoot flavonoids. J. ,oil. Sci. Plant Nutr., 9: 102-115.
Schutzendubel A, Polle A (2002). Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization, J. Exp. Bot., 53: 1351-1365.
Schüepp H, Dehn B, Sticher H (1987). Interaktionen zwischen VA-Mykorrhizen und Schwermetallbelastungen. Angew. Bot., 61: 85-96.
Tonin C, Vandenkoornhuyse P, Joner EJ, Straczek J, Leyval C (2001). Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. Mycorrhiza, 10: 161-168.
Turnau K, Jurkiewicz A, Lingua G, Barea JM, Gianinazzi-Pearson V (2005). Role of arbuscular mycorrhiza and associated microorganisms in phytoremediation of heavy metal polluted sites. In: Trace Elements in the Environment, Prasad MNV, Sajwan KS, Naidu R (eds), CRC Press, Florida, pp.235-252.
Venkateswarlu B, Pirat M, Kishore N, Rasul A (2008). Mycorrhizal inoculation in neem (*Azadirachta indica*) enhances azadirachtin content in seed kernals, World J. Microbiol. Biotechnol., 24: 1243–1247.
Wacker TL, Safir GR, Stephens CT (1990). Effects of ferulic acid on *Glomus fasciculatum* and associated effect on phosphorus uptake and growth of asparagus (*Asparagus officinalis* L), J. Chem. Ecol., 16: 901-909.