Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (3) July-September, pp.295-303/Rahimi
Research Article

THE EFFECT OF ECTOMYCORRHIZAE ON THE CONTENT OF SOME MINERALS IN AHMAD AGHAEI PISTACHIOS TREATED WITH DIFFERENT CONCENTRATIONS OF MAGNESIUM

***Sara Rahimi**
Biology Expert, Animal Science
**Author for Correspondence*

ABSTRACT
Several studies have been carried out to create ectomycorrhizae in sterile conditions and examine the effect of this symbiotic system on plants. However, no study has been conducted to find which ectomycorrhizae and non-mycorrhizal pistachio plants are exposed to different concentrations of magnesium. In this study, ectomycorrhizal plants were aseptically ectomycorrhizae using *Agaricus bisporus* in sterile conditions, and were grown with sterile non-mycorrhizal plants in flasks under four treatments with magnesium sulfate prepared using a half-concentration Hoagland solution. After harvesting the plants, the concentration of calcium, magnesium, phosphorus, iron, potassium, sodium, zinc, manganese and copper was measured in the plants. The intensity of mycorrhizalization in the plants had increased when they were exposed to high concentrations of magnesium. The mineral concentrations in mycorrhizal plants and high concentration of magnesium were increased compared to non-mycorrhizal plants. However, in high concentration of magnesium, ectomycorrhizae prevented high accumulation of magnesium in the shoots. The findings about the role of ectomycorrhizae in nurturing minerals in plants are discussed.

Keywords: *Ectomycorrhizae, Concentration of Minerals, Pistachios, Magnesium Treatment*

INTRODUCTION
In natural conditions, plants make symbiosis with mycorrhizal fungi, but only 3% of the plants have symbiotic relationships (Dell, 2000). There are 6000 species of fungi participating in Ectomycorrhizal symbiosis that are impregnated around the root, and form fungal sheath and Hartig network around the root cells. In symbiotic relationship with the plant, these fungi help absorb water and nutrients. Several experiments have been conducted to induce mycorrhizas and study its effects on the growth of various plants. The experiments have revealed numerous and varied degrees of impregnation, growth and absorption of nutrients, and transferring them to the host plant (Gellier *et al.*, 1984). Different degrees of root impregnation depend on host and fungal symbionts (Baxer and Dighton, 2001).
Ectomycorrhizae, by increasing potassium, iron, and phosphorus for the host plant, increases its metabolism and growth. This condition is manifested by an increase in the concentration of elements in the mycorrhizal plants. There is limited information on the absorption of magnesium and calcium by external Hyphae of ectomycorrhizae, and research has shown that ectomycorrhizal symbiosis can increase or decrease magnesium in the plant tissue or may not even affect it (Martine and Pais, 1997).
In a few studies, it was found that external Hyphae have an important role in magnesium and calcium absorption, but detailed mechanisms are still unknown (Karest and Turkington, 2008). Since overall absorption of nutrients by the ectomycorrhizae depends on both participants' genetics and each has its physiological characteristics, it is necessary to examine the influence of host and fungi in this connection. Pistachio makes symbiotic relationship with endomycorrhizas and ectomycorrhizal fungi. A few researches have been done on ectomycorrhizal symbiotic effect of magnesium nutrition on pistachio, but the effect of the ectomycorrhizal symbiosis of magnesium nutrition on pistachio has not been studied yet.
Furthermore, cultivators in Iran, often plant pistachio without due considerations of soil suitability, and soils are sometimes enriched with magnesium. Another problem is that using groundwater wells in the pistachio cultivation areas has led to decreased water level, which accordingly leads to low quality of groundwater and decreased ratio of calcium to magnesium in irrigation waters of pistachio orchards.

Considering these problems, it is necessary to study the effect(s) of varying degress of magnesium on pistachio plants.

In this study, the pistachio species *Ahmad Aghaei* was the symbiont with *Agaricus bisoprous*, and mycorrhizal and non-mycorrhizal plants were affected by different amounts of magnesium, and effect of this symbiosis was measured on the content of some elements under different magnesium treatments in mycorrhizal and non-mycorrhizal plants.

MATERIALS AND METHODS

First, fungus *Agaricus bisporus* were collected from pistachio orchards of Rafsanjan area, Iran, and then were cultured in Melin-Norcrans agar (MMN) medium, included CaCl2 (0.05 g), NaCl (0.025 g), KH2PO4 (0.5 g), MgSO4 (0.15 g), FeCl3 (1.2 ml), (NH4)2HPO4 (0.25 g), Thiamine Chloride (100 mg), malt extract (3 g), and glucose (10 g). Medium was prepared in 1000 ml of sterile water, and after adding 15 g of agar, the acidity level were set at 5.5. Containers of medium, after fungal culture were placed at room temperature for four weeks under sterile conditions until the fungi started to grow (Laiye *et al.*, 2003). After being soaked in water, pistachio seeds were placed at temperature of 4ºC for two weeks, and then were placed at 0.5 % of hypochloride solution and then in a one percent solution of Tween, and finally were washed with distilled water four times to be sterilized. This was repeated twice, then the seeds were placed in sterilized petri dishes in laboratory temperature in the dark to bud. Peat and perlite were washed four times with tap water and were dried at room temperature and poured in 500 ml flasks and were sterilized at 121º C for 20 minutes. Three weeks after budding, plantlets were entered into 500 ml flasks containing 54 g ml perlite and 6.5 g peat moss (Gellier *et al.*, 1984) and 80 ml Hoagland solution with a concentration of 1.2 besides roots in half of the flasks, fungus pieces with the same size (10) were laid. After 4 weeks of plantlet growth, flasks containing different ratios of magnesium include 1.4, 1, 2, and 4 of the Hoagland solution with 1.2 concentration were treated. Each of two flasks containing mycorrhizal and non-mycorrhizal plants were divided into 4 groups with at least 3 repeats. Every 3-member group of mycorrhizal or non-mycorrhizal plants was treated with 80 ml of magnesium sulfate solution with the above ratios dissolved in sterile water every week. Hoagland solution with a 1.2 concentration included Ca (NO3) 2 (7.38 mg), MgSO4 (1.97mg), KH2PO4 (54.5mg), KNO3 (202 mg), iron citrate (3mg, (CuSO4 (0.1mg), ZnSO4 (0/1mg), molybdic acid (0.1 mg (Na2-EDTA (4 mg) (Bahrampur, 2006). The content of magnesium sulfate in magnesium to calcium ratios of ¼, 1, 2, and 4 was respectively. 0.403, 1.61, 3.22, and 6.44 g/l. Flasks containing plants were kept in the laboratory conditions and were placed under the full light for 9 weeks. After 9 weeks, impregnation to ectomycorrhizae percentage and contents of elements were measured. In order to measure percentage of impregnation in the root, graph paper under a stereoscope was used. To ensure the formation of ectomycorrhizae, transverse incision with a scalpel of roots of some plants that were expected to be microzated was manually prepared. The incisions were made using 1% methylene blue dye in Lactophenol (Laiye *et al.*, 2003). Then the samples (roots and shoots) were placed in a separate furnace at temperature of 550ºC for 5 hours, and were digested by thick HCl. Using this extract, Zerd-Vanadate method, and spectrophotometry, the amount of phosphor was measured, using titration with EDTA method, the amount of magnesium and calcium was measured, using flame photometer model JENWAY the amount of sodium and potassium was measured, using atomic absorption model GBC932AA, the amount of iron, copper, zinc and manganese was measured (Imami, 1993).

The experimental design was as 4 × 2 factorial in which there were two mycorrhizal and non-mycorrhizal levels, and four magnesium concentration levels, conducted in the form of complete randomized design, and all required statistical analyses were performed. Comparing means was conducted using Duncan's test level at 5 percentage level.

RESULTS AND DISCUSSION

The results showed that with the increase of magnesium amount, impregnation of root to ectomycorrhizae increased. in the root cross section, ectomycorrhizae is shown (figure 1).

Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (3) July-September, pp.295-303/Rahimi
Research Article

The results of the impregnation of roots to ectomycorrhizae is also shown (figure 2).

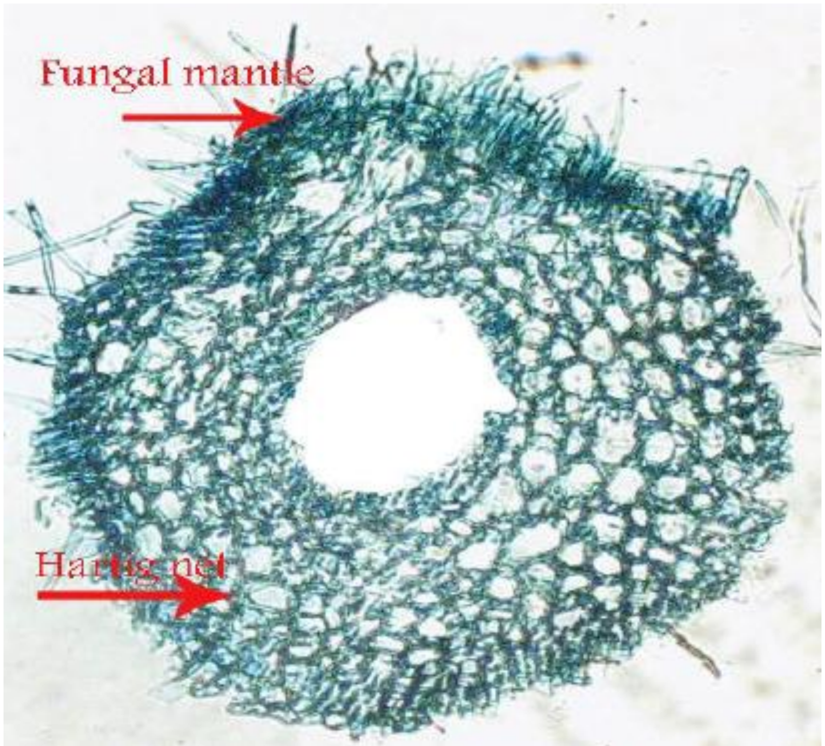


Figure 1: The cross section of the Ectomycorrhizal root fungal mantle, and Hartig network

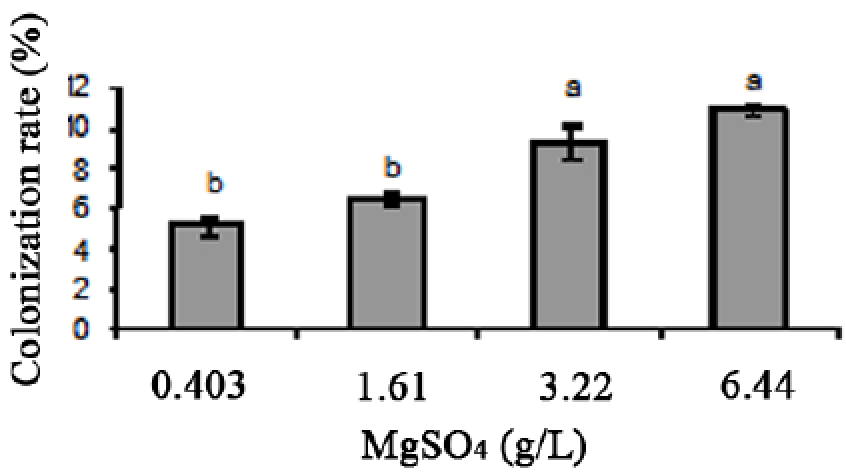


Figure 2: Impregnation percentage of Ahmadaghaei pistachio in different concentrations of magnesium sulfate in the presence of mycorrhizas

When the magnesium content was low (1/61 and 0/403 g magnesium sulfate), calcium content of shoots of mycorrhizal plants increased compared to non-mycorrhizal plants, but it was not significantly different in root (figures 3a, 3b). When the magnesium content was high (6/44 and 3/22 g/l magnesium sulfate), calcium content increased both in roots and shoots of mycorrhizal plants compared to non-mycorrhizal plants (figures 3a, 3b). Magnesium content in shoots of mycorrhizal plants shows a decreasing trend compared to non-mycorrhizal plants. In higher content of magnesium (6.44 and 3.22 g/l magnesium sulfate) magnesium content shows an increase in non-mycorrhizal plants compared to mycorrhizal plants, but these treatments were not significantly different in the root. Decreasing trend of magnesium content in the shoots and increasing trend of magnesium content in the roots were observed in mycorrhizal compared to non-mycorrhizal groups (figures 3c, 3d). The magnesium content in roots and shoots at low concentrations of magnesium (161 and 0.403 g/l magnesium sulfate), showed significant differences between mycorrhizal and non-mycorrhizal plants, and increasing the magnesium content in shoots and roots of mycorrhizal plants was observed compared to non-mycorrhizal plants (figures 3c, 3d). In higher content of magnesium (6.44 and 3.22 g/l magnesium sulfate) it increased Magnesium content in shoots of non-mycorrhizal plants, but was not significantly different in roots (figures 3c, 3d).

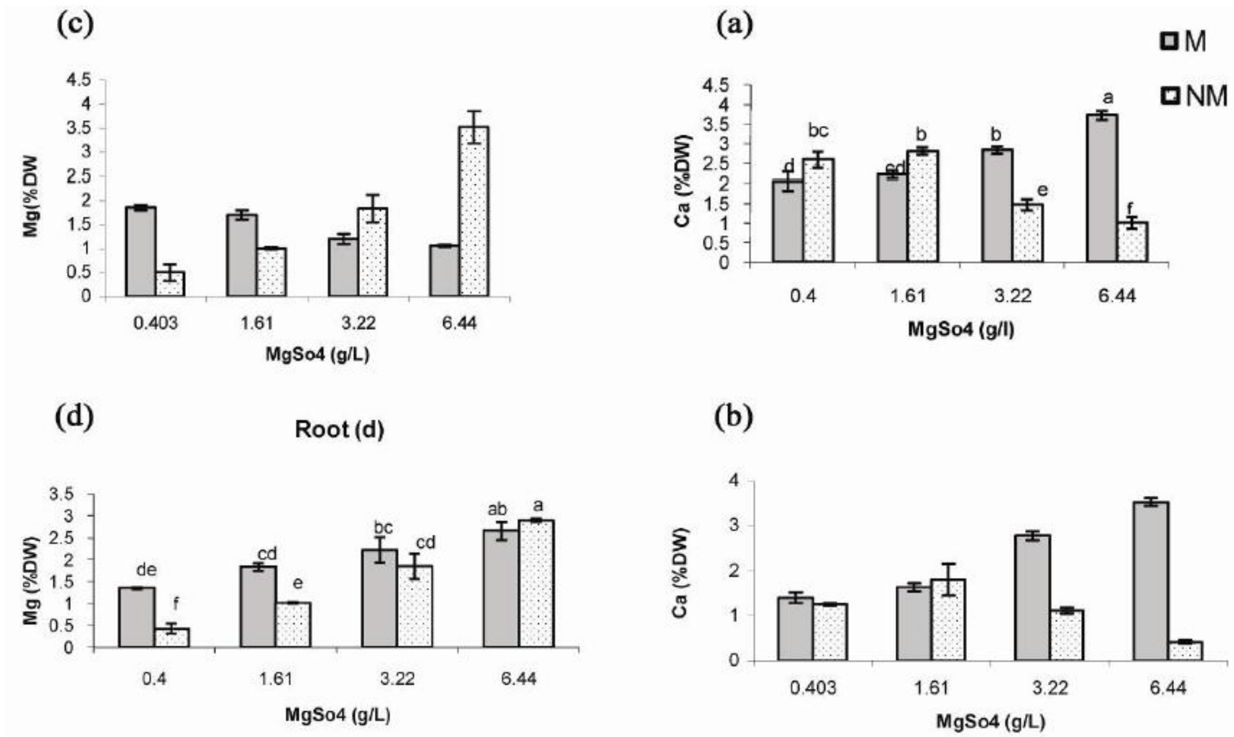


Figure 3: Variation in Calcium content in shoot (a), root (b) and Magnesium content in shoots (c), root (d) of pistachio plant at different concentrations of magnesium sulfate at the presence of mycorrhizas (M) and without it (NM). Different letters in diagram represent the significant difference (P <0.05)

There is increased phosphorus content in shoots and roots in mycorrhizal compared to non-mycorrhizal group. The Phosphorus content in roots and shoots at low Magnesium content (1.61 and 0.403 g/l magnesium sulfate), showed no significant differences between mycorrhizal and non-mycorrhizal plants, but in 6.44 and 3/22 g/l magnesium sulfate treatments showed significant differences both in root and shoot in mycorrhizal and non-mycorrhizal groups, and the increase in Phosphorus was in the mycorrhizal group (figures 4a, 4b). There is an increased Potassium content in shoots and roots of mycorrhizal plants compared to non-mycorrhizal plants and decreased potassium content in non-mycorrhizal plants compared to mycorrhizal plants (figures 4c, 4d). Potassium content in roots and shoots at low magnesium content (1.61 and 0.403 g/l magnesium sulfate), showed a significant difference between mycorrhizal and non-mycorrhizal groups, and a decrease in potassium content in mycorrhizal plants was observed both in roots and shoots compared to non-mycorrhizal plants. Increased content of potassium in high magnesium content (6.44 and 3.22 g/l magnesium sulfate) in both shoots and roots in mycorrhizal group was shown compared to non-mycorrhizal group (figures 4c, 4 d). Iron content in roots and shoots in all treatments with magnesium sulfate showed significant difference between mycorrhizal and non-mycorrhizal groups, and increased iron in roots and shoots of mycorrhizal plants was observed compared to non-mycorrhizal plants (figures 4e, 4f).

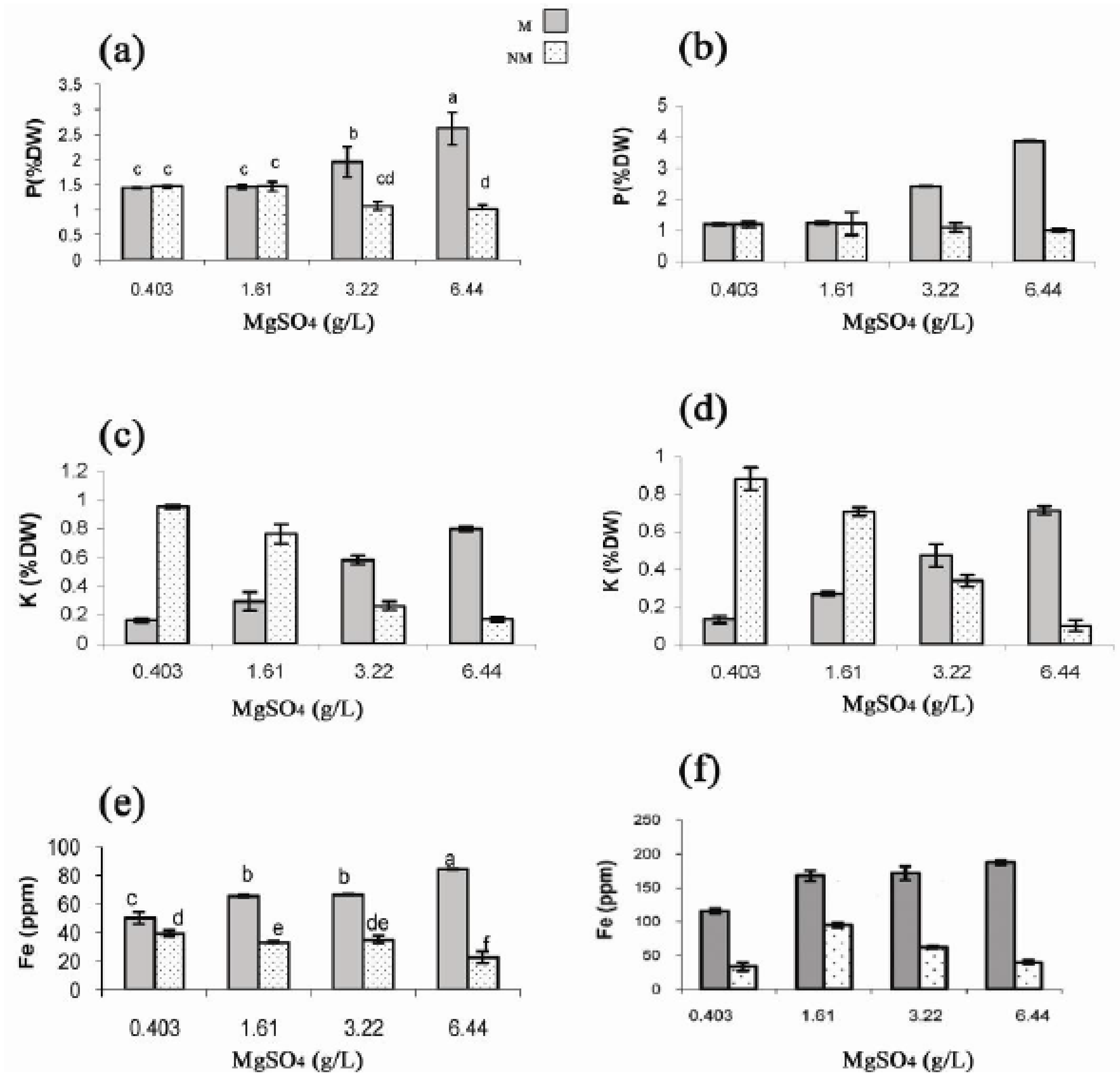


Figure 4. Variation in Phosphorous content in shoot (a), root (b) and Iron content in shoots (c), root (d) of pistachio plant at different concentrations of magnesium sulfate at the presence of mycorrhizas (M) and without it (NM). Different letters in diagram represent the significant difference (P <0/05)

Sodium content in low levels of magnesium (1/61 and 0/403 g/l magnesium sulfate) showed no significant differences in both root and shoot in mycorrhizal and non- mycorrhizal groups. Increase sodium content in high content of magnesium (6.44 and 3.22 g/l magnesium sulfate) in shoots and roots were observed in mycorrhizal plants compared to non-mycorrhizal plants (figures 5a, 5b). At low levels of magnesium (1.61 and 0.403 g/l magnesium sulfate), there is no significant difference in roots and shoots, but in higher magnesium content (6.44 and 3.22 g/l magnesium sulfate), sodium content increased in both roots and shoots (figures 5a, 5b).

Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (3) July-September, pp.295-303/Rahimi
Research Article

Zinc content in shoots in 0.403 g/l magnesium sulfate showed no significant differences between mycorrhizal and non-mycorrhizal groups, but it showed a significant difference in the root, as a result of which mycorrhization in this treatment led to reduced zinc (figures 5c, 5d). Zinc in 1.61 g/l of magnesium sulfate treatment showed significant differences between mycorrhizal and non-mycorrhizal groups, and decreased zinc content in the shoots and roots of mycorrhizal plants was observed compared to the non-mycorrhizal plants.

With a high content of magnesium (6.44 and 3.22 g/l magnesium sulfate) in the shoots and roots, zinc content of mycorrhizal plants showed increase compared to non- mycorrhizal plants (figures 5c, 5d).

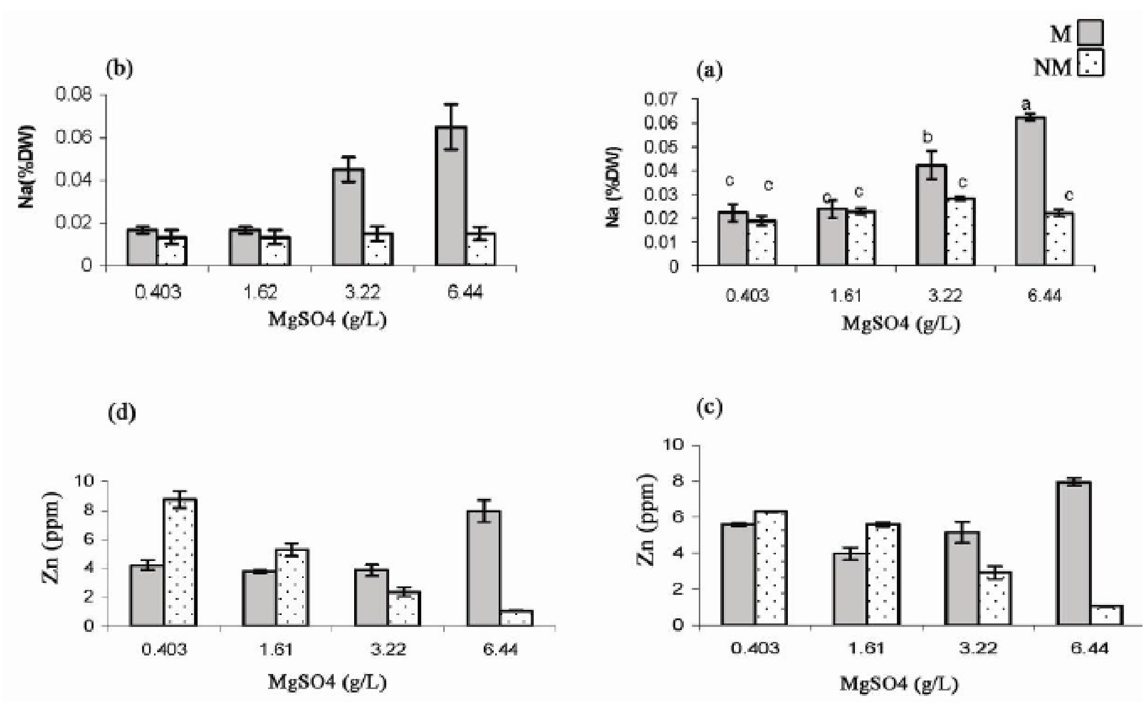
Copper content in the shoots of mycorrhizal plants showed an increasing trend compared to the non-mycorrhizal plants. In 0.403 g/l magnesium sulfate treatment, in the shoots, there was a significant difference between mycorrhizal and non-mycorrhizal groups, and reduced copper content in the shoots was observed in mycorrhizal plants compared to non-mycorrhizal plants, but there was no significant difference in the root.

Copper in 1,61 g/l of magnesium sulfate treatment showed no significant difference between mycorrhizal and non-mycorrhizal groups, but there was a significant difference in the root. With a high content of magnesium (6.44 and 3.22 g/l magnesium sulfate) in shoots and roots, zinc content of mycorrhizal plants showed increase compared to non-mycorrhizal plants (figures 5e, 5f).

Manganese content in shoots in 0.403 g/l magnesium sulfate showed significant differences between mycorrhizal and non-mycorrhizal groups in shoots and roots, and reduced manganese content in the shoot was observed compared to non-mycorrhizal plants.

Yet, there was an increased content of manganese in the root in mycorrhizal compared to non-mycorrhizal group (figures 5g, 5h manganese content in 1.61 g/l of magnesium sulfate treatment showed no significant differences between mycorrhizal and non-mycorrhizal groups both in the roots and shoots.

At high magnesium content (6.44 and 3.22 g/l of magnesium sulfate), and increased content of manganese in the shoot of mycorrhizal plants was observed compared to non-mycorrhizal plants (figures 5g, 5h)



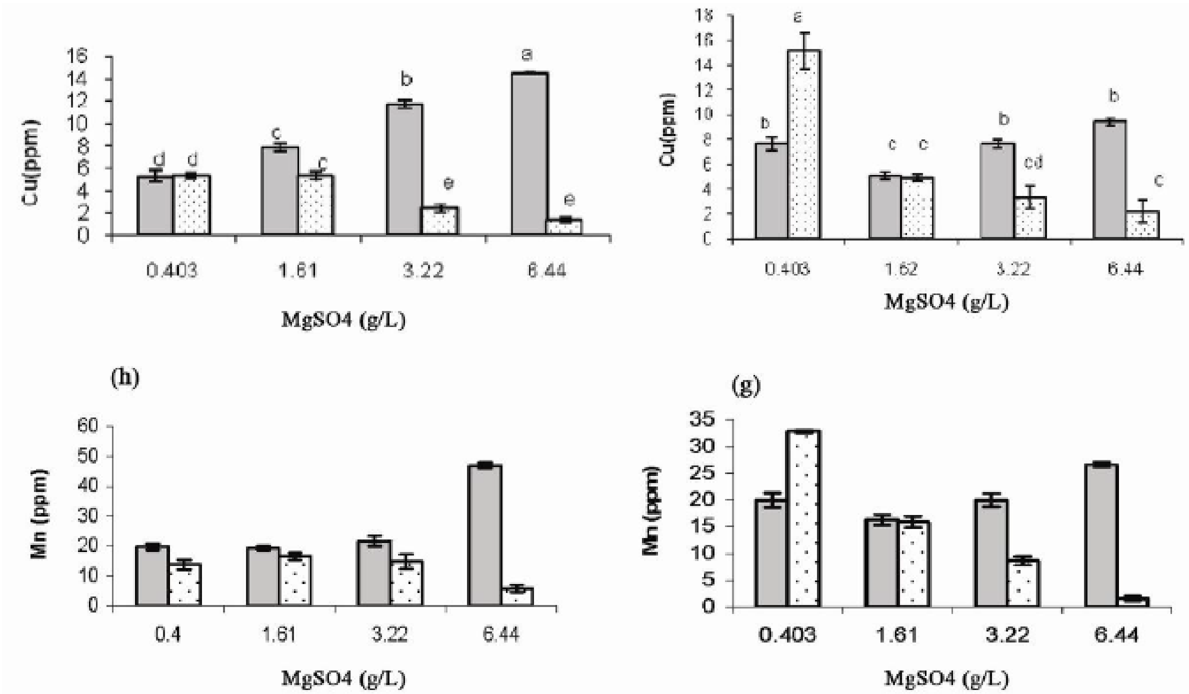


Figure 5. Variation in Sodium content in shoot (a), root (b), Zinc content in shoots (c), root (d), Copper content in shoots (e), root (f), and manganese content in shoots (g), root (h) of pistachio plant at different concentrations of magnesium sulfate at the presence of mycorrhizas (M) and without it (NM). Different letters in diagram represent the significant difference (P <0.05)

Conclusion

According to the results of the effect of ectomycorrhizae on feeding of some elements, increased magnesium indicated that the mycorrhizas was able to prevent moving large amounts of magnesium to shoot and keep magnesium in rows around the plant root, so as increased impregnation of root to mycorrhizas can be considered as ectomycorrhizal system reaction in order to fix the problem of excessive magnesium content. However, there is a question why magnesium is concentrated in the roots of fungi. Ectomycorrhizal mechanisms preventing excessive magnesium content involve (a) bonding to the fungal mantle; (b) reducing apoplasty mobility as a result of the hydrophobic effect of fungal mantle; (c) being chelated by organic acids; and (d) bonding to external Hyphae (Kayama *et al.,* 2005). Increasing magnesium reduced amount of some elements such as calcium and potassium in non-ectomycorrhizal plants. High magnesium content may decrease the growth and prevent uptake of elements such as calcium and potassium. High level of magnesium also prevents root growth and inhibits enzyme activity of mitochondria (Kayama *et al.,* 2005). Low level of these elements in non-mycorrhizal plants could be due to the competitive nature of these three elements (viz. calcium, magnesium, and potassium). These three elements can take the same positions and increasing magnesium can intensify the deficiency of these three elements (Mangal and Karkeby, 1993).

In the present study, *Agaricus bisporus* increased pink theme of calcium content of the plant in high concentrations of magnesium. This indicated the positive role of these fungi in pistachio gardens. Whereas calcium deficiency, with changes in plasma membrane permeability, decrease the influence ability of ions, high content of magnesium in the root may affect the amount of potassium and lead to a drop of potassium in the plant. ectomycorrhizae increases potassium in plant which may probably increase photosynthesis and ATP production, since potassium deficiency can reduce photosynthetic capacity (Mangal and Karkeby, 1993).In this experiment, phosphorus content also increased in mycorrhizal plants compared to non-mycorrhizal plants. In ectomycorrhizal plants with low percentage impregnation, high magnesium may reduce phosphorus and potassium content in root. The role of ectomycorrhizae associated with increasing levels of nutrients in the host (Kayama *et al.*, 2005) confirms the obtained results. By chemical changes in the rhizosphere including acidification and producing metal chelates, ectomycorrhizae increases solubility of inorganic phosphorus, and thus increase its uptake and transfer to the host plant (Wallander, 2000).

By measuring iron in non-mycorrhizal plants, it was shown that the amount of iron in the roots and shoots decreased. Still, despite the presence of iron in the growth medium, non-mycorrhizal plants failed to provide adequate iron, which is a conditions suggesting several possibilities such as insolubility of iron, combination with chemical foundations, immovability, or competition with other elements. However, this requires further research. Ectomycorrhizae produce siderophores that increase the root tendency absorb iron when magnesium content is too high (Marschener and Dell, 1994). Increase in magnesium can reduce potassium competitive effects and sodium can be increased in the plant. The amount of sodium showed increase at high magnesium content in shoots and roots of mycorrhizal plants. The amount of sodium in the roots was higher than that in the stems. Ectomycorrhizae keep sodium in its Hyphae and Myceliums. This situation, the concentration of sodium in the roots of pistachio, is also shown in vesicular-arbuscular symbiosis (Bahrampur, 2006). However, further research should clarify whether this excess of sodium is concentrated in the fungus mycelium or root tissues?

In the present study, manganese, zinc and copper content in mycorrhizal plants showed a significant increase compared to non-mycorrhizal plants. Meristem tissues need manganese, which is a main part of the electron donor at photosystem II in water photolysis. Copper and Zinc have structural roles in many enzymes and increase enzyme activity (Mengel and Kirkby, 1993).

In symbiosis of *Pinus virginiana* plants with ectomycorrhizae, Mn and Zn content in roots and shoots of mycorrhizal plants increased (Miller and Rudolph, 1986), which is consistent with results of this experiment. Reduction of manganese, zinc and copper in non-mycorrhizal plants could be due to the competitive effect of magnesium and manganese, zinc and copper (Marschner, 2005).

Summation

The results indicated that mycorrhization in high magnesium concentration in the environment can increase in the amount of phosphorous, potassium, calcium, iron, sodium, manganese, zinc and copper in mycorrhizal plants compared to non-mycorrhizal plants. Reduction in amounts of some of the above elements in mycorrhizal plant and increased amount of these elements in non-mycorrhizal plants were observed, especially when magnesium levels were low. This might have happened because in non-mycorrhizal plants, this amount of magnesium meets the plant needs, and does not create a stressful condition. However, in mycorrhizal plants, although this magnesium content meets the plant needs, because the plant has spent a lot of energy for this symbiosis and spent 10-20% of its net photosynthesis for the formation of this symbiosis, and because fungi, too, need high carbohydrate content, we observed a decrease in the content of these elements in the plant.

REFERENCES

Bahrampur M (2006). Effect of magnesium and calcium on pasticcio almond-type considering the relationships between magnesium and mycorrhizal condition of the plant. MA thesis, Kerman: Shahid Bahonar University.

Baxer JW and Dighton J (2001). Ectomycorrhiza diversity alters growth and nutrient acquisition seedings in host-symbiont culture condition. *New Phytologist* **153** 139-199.

Dell B (2002). Role of mycorrhizal fungi in ecosystems. *Journal of Central Michigan University (CMU)* **1** 47-54.

Emami A (1993). *Techniques in Plant Anatomy* (Soil and Water Research Center, Tehran University Press) 982.

Gellier B, Letouze R and Steullu DG (1984). Micro propagation of Brich and mycorrhizal formation *in vitro*. *New Phytologists* **97** 591-599.

Karst Jones MD and Turkington R (2008). Ectomycorrhizal colonization and intraspecific variation in growth response of Lodge pole pine. *Plant Ecology*. DI10.1007/s 11258-008-9443-9.

Kayama M, Quoreshi AM, Uemura S and Koike T (2005). Difference in growth characteristics and dynamics of elements absorbed in seedlings of three Spruce species raised on serpentine soil in northern Japan. *Annals of Botany Company*.

Laiye QU, Quoreshi AM, Iwase K, Tamai Y, Funada R and Koike T (2003). *In Vitro* ectomycorrhizal formation on two larch species of seedlings with six different fungal species. *Eurasiana. For.Res* **6** 65-73.

Legget JE and Gilbert WA (1999). Magnesium uptake by soybean. *Plant Physiology* **44** 1182-1186.

Marschener H and Dell B (1994). Nutrient up take in mycorrhizal symbiosis. *Plant and Soil* **159** 89-102.

Marschner H (2005). *Mineral Nutrition of Higher Plants* 2nd edition, Translated by Kholdebarin B and Eslamzadeh T (Shiraz: Shiraz University Press).

Martin A, Casimiro A and Pais MS (1997). Influence of mycorrhization physiological parameters of micro propagated *Castahea sativa* Mill. plants. *Mycorrhiza* **7** 161-165.

Mengel K and Kirkby EA (1993). *Principles of Plant Nutrition* 2nd edition, translated by Salardini A and Mojtahedi M (Tehran: Tehran Univeristy Press).

Miller FA and Rudolph ED (1986). Uptake and distribution of manganese and zinc in *Pinus virginiana* seedlings infected with *Pisolithus tinctorius*. *OHIO Journal of Science* **86**(4) 22-25.

Mukerji KG and Chamola BP (2003). Compendium of mycorrhizal research. *In: Concepts in Mycorrhizal Research* (Kluwer Academic Publisher, London) 373.

Quoreshi AM and Khasa DP (2008). Effectiveness of mycorrhizal inoculation in the nursery on root colonization, growth, and nutrient uptake of aspen and balsam poplar. *Biomass and Bioenergy* **32** 381-391.

Wallander H (2000). Up takes of P from apatite by *Pinus sylvestris* seeding colonization by different ectomycorrhizal fungi. *Plant and Soil* **218** 249-256.