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Effects of endomycorrhizal fungi and drought stress on nutrient acquisition of Walnut (*Juglans regia* L)

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ABSTRACT:

Arbuscular Mycorrhizae (AM) are associated with higher plants by a symbiotic association, and benefit plants in uptake of phosphorus, nitrogen and zinc. Walnut is grouped under the drought sensitive crops. Mycorrhizal fungi is the symbiotic relationship between plant roots and beneficial fungi, confering the stress tolerance in the host plants. This stress tolerance improved due to Arbuscular Mycorrhizal fungi (AM fungi) colonization can be credited to enhanced mineral nutrition. The effects of Arbuscular Mycorrhizal fungi (AMF) *Glomus mosseae* (Nicol and Gerd) Gerdemann and Trappe, *Glomus etunicatum* Becker and Gerdemann and a combination of two fungi species *(Glomus* mix*)* inoculation on growth and mineral acquisition of three genotypes of *Juglans regia* L. grown under drought stress condition was studied. Drought stress was applied with holding irrigation for 20 days in the middle of plant growth period. Plants were grown in sandy soil in a greenhouse. The contents of phosphor (P), nitrogen (N) and zinc (Zn) were higher in Mycorrhizal (M) than Non Mycorrhizal (NM) plants control and drought conditions. Generally, it can be said that mycorrhizal plants of *Juglans regia* showed higher tolerance toward drought stress than NM plants and their growth improved by AMF colonization. The results indicated that all the physical parameters were enhanced with applying AM fungus.

Keywords:
Arbuscular mycorrhizal fungi, Nutrient uptake, Drought, *Juglans regia*.

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INTRODUCTION

Advantageous impacts of AM organisms as effective scavengers of nutrients and as bio-control agents have been well known in agricultural production. Be that as it may, the usage of these fungi is constrained by the absence of accessibility of inoculum in huge amount. Their most reliable and significant dietary impacts are to improve uptake of nutrient supplements, for example, phosphorus, copper and zinc (Manjunath and Habte, 1988). Arbuscular mycorrhizal fungi have their most noteworthy impact when a host plant related with them, which is of insufficient in phosphorus (Koide and Scheiner, 1992). Mycorrhizal fungi can enhance growth in various farm crops harvests (Mosse, 1973; Tinker, 1975).

Sanni (1976) reported the raise in the growth of rice plants after treatment with *Giaspora gigentia*. Usage of AM inoculum in fruits like banana and papaya farming was reported by farmers of two Indian towns and the farmers in these towns were additionally persuaded about its benefits (Mohandas *et al*., 2004). Arbuscular Mycorrhizal Fungi (AMF) are beneficial symbiotic fungi known to increase the nutrient uptake in plants, particularly phosphorous, sulfur, copper and zinc (Abbott *et al.*, 1994). AMF also known for their role in enhancing root architecture and surrounding soil structure. AMF have been found to protect the roots from pathogen and nematodes (Bagyaraj and Sreeramulu, 1982). The mycorrhizal uses of the indigenous fungi compared with a prerequisite for phosphate by the plants that were colonized by AM fungi naturally present in the soil proportionate to a large portion of that required by non-mycorrhizal plants (Gazey *et al*., 2006).

Doud *et al*. (2002) found that addition of enhanced supplements, for example, water, inorganic nutrient solution without phosphorus and fish protein, was pointless however heavy inoculums of AM fungi in a way promptly utilized as a amendment to plant plotting media for the production of vegetable seedlings. To get greatest agricultural profit, soil inoculation with reasonable kind of AM fungi is fundamental. Consequently, the best way to culture and keep up arbuscular mycorrhizal fungus production, the utilization of AM inoculum that is delivered by extra use of these fungi ought to turn out to be monetarily attainable. Therefore, pot experiment inoculum production was done in the present investigation for assessment of the impact of the indigenous mycorrhiza on the growth and nutrition of walnut plants.

The plants that normally encourage the higher colonization of AM fungi can be commonly utilized as stock plants (Parmita, 2005). Stock plants to culture the AM fungi for inoculum to be used as bio manure were chosen so as to expand agricultural yields just as to limit the utilization of chemical fertilizer and in this manner diminish the ecological pollution. Phosphorus is one of the key macronutrient required for plant growth. It assumes a significant job in energy transfer through the formation of energy rich phosphate esters and is additionally a basic part of macromolecules, for example, nucleotides, phospholipids and sugar phosphates (Marschner, 1995). Much of the inorganic phosphate applied to soil as a fertilizer is rapidly converted to unavailable forms with low solubility. Soluble phosphorous is released from insoluble phosphates by a variety of solubilization reactions involving rhizosphere. microorganisms (Kapoor *et al*.,

Table 1. Characterization of soil used for the experiments

Soil texture	Clay (%)	Silt (%)	Sand (%)	Electrical conductivi-ty (Ds/m)	pH	P (mg /kg)	K (mg /kg)	Organic carbon (%)	Total nitrogen (%)	Fe (mg/ kg)	Mn (mg/ kg)	Zn (mg/ kg)	Cu (mg/ kg)	Depth of sampling
Silty loam	13	31	56	0.62	7	4.94	204 2	0.5	0.05	1.66	8.79	1.88	1.3	0-30 cm

Table 2. Variance analysis of nutrient elements concentration

S. No	Value sources	df	Mean squares		
			N	P	Zn
1	Mycorrhizae	3	8.59**	0.58**	0.12**
2	Drought stress	1	4.76**	0.201**	0.04*
3	Genotype	2	0.492**	0.17**	0.09n.s
4	Mycorrhizae × stress	3	2.09**	0.01*	0.004n.s
5	Mycorrhizae × genotype	6	0.498**	0.089**	0.009n.s
6	Stress × genotype	2	0.382**	0.004n.s	0.05**
7	Fungi × stress × genotype	6	0.782**	0.02**	0.022*
8	Error	52	0.09	0.0036	0.0094
9	Corrected total	71	0.65	0.042	0.016
10	CV		5.7	3.56	9.64

* and ** : Significant at 5% and 1% levels respectively

1989).

The uptake of phosphorous is more in mycorrhizal plants. Cultivation with Phosphate Solubilizing Microorganisms (PSM) may help to solubilize soil phosphate, just as phosphorus from rock phosphate. Soluble phosphate discharged by the movement of PSM, can effectively be taken up by mycorrhizal roots (Kapoor *et al*., 1989). The rate of assimilation of phosphate by roots is a lot higher than the rate of soil phosphate diffusion, which results in the arrangement of a phosphate depletion zone at the root level and therefore constrains the supply of phosphorus to the plant. The developing plant root makes a phosphate depletion zone brought about by contrastingly high plant phosphate uptake and low soil-based phosphate dispersion rates.

Table 3. Means comparison of mycorrhizae on three nutrient elements concentration

S. No	Treatments	N	Zn	P
1	Control	4.74c	0.88b	1.45d
2	*G. mosseae*	5.07b	1.04a	1.67c
3	*G. etunicatum*	4.97b	1.06a	1.88a
4	*Glomus* mix	6.28a	1.03a	1.77b

The extra-radical mycelium of arbuscular mycorrhizal fungi grows past the phosphate exhaustion zone, achieving another pool of dissolvable phosphate (Smith and Read, 1997). The significant capacity of AM fungus is phosphate uptake, in light of the fact that it encodes a phosphate transporter gene. Harison and Buuren (1995) explored a procedure for phosphate transport by recognizing a complementary DNA (cDNA) that encodes a trans membrane phosphate transporter named GvPT from *G. versiforme*. GvPT expression was confined to the external hyphae of *G. versiforme* during mycorrhizal interaction, this being the start site of phosphate uptake from the soil. Chellappan (2000) confined a phosphate transporter gene from *G. deserticola* and additionally demonstrated the job of AM organisms, in the uptake of phosphate. Plant roots can take up nitrogen from the soil both as nitrate or ammonium.

GS/GPGAT pathway is currently the recognized pathway in higher plant roots, whereas nitrogen assimilation in a few ectomycorrhizal organisms happens conspicuously through the GDH pathway

Table 4. Means comparison of drought stress on three nutrient elements concentration

S. No	Drought stress	N	Zn	P
1	Control	5.01[b]	0.98[b]	1.64[b]
2	Stress	5.52[a]	1.03[a]	1.75[a]

Table 5. Means comparison of genotype on three nutrient elements concentration

S. No	Genotype	N	Zn	P
1	Chandler	5.8[a]	1.3[a]	1.84[a]
2	Serr	5.11[c]	1.03[c]	1.6[b]
3	Panegine20	5.28[b]	1.18[b]	1.74[c]

(Busse and Ellis, 1985). The absorption of nitrogen by mycorrhizal fungi/roots is pretty much a similar example as assimilation by plant roots. Mycorrhizal fungi are known to create both nitrate reductases and enzymes for assimilation of ammonium.

Water shortfall is a significant abiotic factor constraining plant development and yield in numerous zones on the earth that is progressively topical in view of environmental change and water deficiencies (Kwapata and Hall, 1995). A few eco-physiological investigations have shown that arbuscular mycorrhizal symbiosis frequently brings about modified rates of water movement, through and out of the host plant, with ensuing impacts on tissue hydration and plant physiology. It is currently acknowledged that the commitment of AM beneficial interaction to plant drought tolerance is the consequence of physical, physiological and cellular impacts (Cooper and Losel, 2001).

Allen and Allen (1981) demonstrated a conceivable role of AM organisms in water uptake by the host plant. Also, organic carbon got from photosynthesis is moved to these symbioses, which are bio-trophic microbes maintaining the growth of spores and fruit bodies in most mycorrhizae types by translocation of the content to the developing edges of the extradural mycelium (Allen and Boosalis, 1981). Phosphorous has a significant role as a energy carrier during photosynthesis (Barea and Azcon-Aguilar, 1982). Accordingly, AM fungi may work as a metabolic sink making basipetal of photosynthetic roots, in this way giving stimulus to more noteworthy photosynthetic activity. Likewise, improved levels of cytokines could raise photosynthetic rates by stomal opening impacting ion transport and directing chlorophyll levels.

Arbuscular mycorrhizae assume significant job in terrestrial eco-systems, for example, fields, where they impact plant community nutrient cycling (Graha and Syversen, 1984). Mycorrhizal interaction upgrades

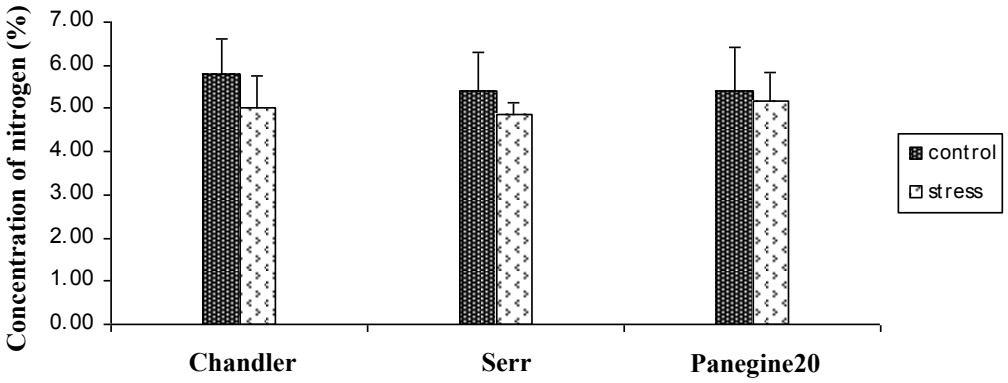

Figure 1. Interaction effect of drought stress and genotype on amounts of nitrogen

Table 6. Means comparison of drought stress and genotype on amounts of zinc

S. No	Treatments	Genotype			Means
		Chandler	Serr	Panegine20	
1	Control	1.02±0.02[b]	0.12±0.01[b]	0.13±0.01[b]	0.42
2	Stress	0.997±0.03[b]	1.1±0.01[a]	0.995±0.02[b]	1.03
3	Means	1	0.61	0.562	0.725

plant development and profitability by expanding nutrient uptake (Al-Karaki *et al*., 1998). They likewise grant different advantages to plants including upgraded enzymatic production (Kramer and Boyer, 1995), expanded rate of photosynthesis (Lin *et al*., 1996), increased nitrogen fixation by symbiotic or interactive N_2-fixing bacteria (Menge, 1983), osmotic alteration under drought stress (Ruiz-Lozano, 2003), increased pest resistance (Whipps, 2004), resilience to different abiotic stress factors (Nemec and Meredith, 1981) and improving soil aggregation (Rillig and Mummey, 2006; Pacovsky and Fuller, 1988) and hence improved soil physical properties and stability. Hence, the present study was undertaken to access the impact of mycorrhizal interaction on nutrients uptake and drought stress.

MATERIALS AND METHODS

Preparation of soil and inoculation of prepared soil

Three genotypes of walnut (*Juglans regia L*.) seeds includes: "Chandler", "Serr" and "Panegin20" were obtained from seed center of Karaj and they have been kept at 4°C for 15 days to germinate and sterilized with kaptan 0.2% (v/v) for 15 min. Prepared soil contained sand, clay and silt (Table 1). Sterilized seeds were planted in soil and inoculum of mycorrhizal fungi (10 g), was laid around the seed. The pots were maintained under optimal conditions for about two months before long term water stress treatments were begun. After two months when seedlings were grown smoothly, water stress is carried out in the middle of growth time through holding irrigation for 20 days.

Preparation of mycorrhizal inoculums

Inocula of the arbuscular mycorrhizal fungi used were isolates of *Glomus mosseae* (Nicol and Gerd) Gerdemann and Nicolson (1963), Trappe and *Glomus etunicatum* Becker and Gerdemann and *Glomus* mix, from soil and water research institute of Tehran. All mycorrhizal inocula consisted of sand, spores and mycelium.

Estimation of total nitrogen

The total nitrogen was determined by the conventional micro-Kjeldhal method described by Sadasivam and Manicham (1996).

Table 7. Means comparison of mycorrhizae and drought stress on amounts of nitrogen

S. No	Treatments	Drought stress		Means
		Control	Stress	
1	Control	4.59±0.24[f]	4.88±0.4[e]	4.73
2	*G. mosseae*	4.43±00.34[f]	5.7±0.17[c]	5.06
3	*G. etunicatum*	4.57±0.54[f]	5.38±0.032[d]	4.97
4	*Glomus* mix	6.44±0.15[a]	6.12±0.69[b]	6.28
5	Means	5.007	5.52	5.26

Table 8. Means comparison of mycorrhizae and genotype on the amounts of nitrogen

S. No	Treatments	Genotype			Means
		Chandler	Serr	Panegine20	
1	Control	5.06±0.24^{cde}	4.64±0.38^{fg}	4.51±0.14^{g}	4.73
2	*G. mosseae*	5.16±0.35^{cd}	5.08±0.72^{cde}	4.96±1.01^{cdef}	5.06
3	*G. etunicatum*	4.74±0.66^{efg}	4.85±0.61^{defg}	5.33±0.39^{c}	4.97
4	*Glomus* mix	6.64±0.38^{a}	5.89±0.54^{b}	6.31±0.35^{a}	6.28
5	Means	5.4	5.115	5.27	5.26

Estimation of phosphorus and zinc

Concentration of phosphorous and zinc were determined with digested and ICP according to the method mentioned by Osonubi *et al*. (1991). Experimental design was a factorial randomized with five factors (fungi, bacteria, drought stress levels, genotypes of walnut and time) and four replications.

Statistical procedure

Analysis of variance was performed on all data sets. Duncan test with probability of 0.05 was used to show significant differences between treatments. All data are presented as means ± standard error (SAS, 2006).

RESULTS AND DISCUSSION

The results showed that drought stress significantly affected the amounts of nutrient as given in ($P<0.05$) Table 4. Highest concentration of nitrogen, zinc and phosphorus appeared under stress conditions. Also, the results showed that mycorrhizal significant effects on measured elements was efficiency at $P<0.05$ as given in Table 3. Three species mycorrhizae have significant effects on amounts of three nutrient elements that were measured. *G. etunicatum* had the highest effects on amounts of phosphorus. *Glomus* mix had the same effects on amounts of nitrogen and three species of mycorrhizae have significant effects on amounts of zinc in treated plant as compared with control. In all factors, control plants have lowest effect among all the treatments. In three genotypes, amounts of nitrogen and zinc and phosphorus were highest in Chandler, and then in Panegin20 followed by Serr (Table 5).

The highest of zinc appeared under application of three species mycorrhizae and serr genotype under drought stress conditions (Table 6). Seedling of Chandler genotype treated with *Glomus* mix under well water conditions have highest amounts of nitrogen in their leaves (Table 7, 8 and 9). As of phosphorous, highest amounts appeared on seedling of Serr genotype treated with *G. etunicatum* and stress conditions (Table 10, 11 and 12).

Mosse and Herper (1975) discovered that the degree of root intensity is higher in mycorrhizal non-phosphorus rice plant than in mycorrhizal rice plant

Table 9. Means comparison of drought stress and genotype on the amounts of nitrogen

S. No	Drought stress	Genotype			Means
		Chandler	Serr	Panegine20	
1	Control	5.02±0.81^{a}	4.84±0.93^{b}	5.15±1.007^{b}	5.52
2	Stress	5.78±0.73^{cd}	5.38±0.28^{d}	5.4±0.66^{bc}	5
3	Means	5.4	5.11	5.27	5.26

Table 10. Means comparison of mycorrhizae and drought stress on the amounts of phosphorus

S. No	Treatments	Drought stress		Means
		Control	Stress	
1	Control	1.38±0.05^{f}	1.53±0.01^{e}	1.45
2	*G. mosseae*	1.64±00.01^{d}	1.71±0.07^{c}	1.67
3	*G. etunicatum*	1.8±0.26^{b}	1.95±0.17^{a}	1.87
4	*Glomus* mix	1.74±0.08^{c}	1.8±0.14^{b}	1.77
5	Means	1.64	1.74	1.69

accepting added phosphorus. The decrease of mycorrhizal infection in the presence of added phosphorus is attributable to a self-regulatory action of plant disposing of the mycorrhizal fungi when its phosphorus necessity is more than that fulfilled (Hayman, 1982). Gazey *et al*. (2006) likewise stated that the mycorrhizal benefit was autonomous of the plant-available phosphorus in the soil. Further, there was no extra advantage of inoculation on plant development other than of the expanded phosphorus uptake. Additionally, when phosphorus is provided at high amounts, as normally done when developing plants in soil where AM organisms are missing, at that point it can cause nutritional disorder as a result of its hostile co -operations with different nutrients or it represses mycorrhizal formation (Lambert and Baker, 1979).

Plants associated with AM fungi are capable to get phosphorus when they are later planted into low phosphorus soil. It was also proposed that infection with VA fungus can build phosphorus and chemical phosphate. Phosphorus and nitrogen uptake by walnut plants were likewise assessed to determine the impact of indigenous VA mycorrhiza, and found that VAM organisms enormously improves phosphorus and nitrogen uptake by walnut plants growth. Enhancement of phosphorus uptake by mycorrhizal plants was also reported for by Pacovsky (1989). Draft and Nicolson (1966) studied the effect of different amounts of phosphate to soil and sand, and found that the mycorrhizal infection and the beneficial impacts of VA mycorrhiza on the host plant were inversely related with the measure of accessible phosphate. Murdoch *et al*. (1967) and Jakobson (1980) also arrived the same conclusion by adding phosphates of shifting accessibility to soils. From soil examination results, it was found that AM inoculation significantly enhanced the supplements than non-inoculated soil. From our examination, it was likewise seen that AM inoculated

Table 11. Means comparison of mycorrhizae stress and genotype on the amounts of phosphorous

S. No	Treatments	Genotype			Means
		Chandler	Serr	Panegine20	
1	Control	1.48±0.08^{f}	1.47±0.06^{f}	1.42±0.1^{f}	1.45
2	*G. mosseae*	1.7±0.08^{cd}	1.68±0.05^{cde}	1.64±0.01^{de}	1.67
3	*G. etunicatum*	1.92±0.04^{b}	2.1±0.04^{a}	1.6±0.14^{e}	1.87
4	*Glomus* mix	1.87±0.1^{b}	1.72±0.09^{c}	1.73±0.09^{c}	1.77
5	Means	1.74	1.74	1.59	1.69

Table 12. Means comparison of drought stress and genotype on the amounts of phosphorous

S. No	Drought stress	Genotype			Means
		Chandler	Serr	Panegine20	
1	Control	1.68±0.18^{bc}	1.69±0.24^{b}	1.56±0.19^{d}	1.64
2	Stress	1.81±0.18^{a}	1.8±.23^{a}	1.63±0.08^{c}	1.74
3	Means	1.74	1.74	1.59	1.69

soil turned out to be increasingly fertile with no sort of other chemical fertilizers.

Nutrient uptake is greatly enhanced by mycorrhizal fungi. Mycorrhizae are in most cases not essential for plant life, especially if soil nutrient levels are high (in fact, mycorrhizal associations may not even form under such conditions). However, this is rarely the case, so mycorrhizae are almost always present and beneficial. When nutrient supply is poor, the percentage of increase in growth resulting from mycorrhizae can be very substantial (e.g. over 900% in western red cedar). Mycorrhizae are especially important in the absorption of phosphorus. The fungus extends the zone of nutrient absorption out several centimeters beyond each root. In addition to promoting nutrient uptake, mycorrhizae help protect roots from disease and can assist in water uptake.

The uptake of macro-nutrients in tree roots under environmental conditions and the long-range transport of the elements in the shoot is influenced by a number of processes such as ion exchange processes, physiological state of the plant, mycorrhization, formation of complexes and transpiration (Bradfield, 1976; Clarkson, 1984; Gülpen *et al*. 1995; Lüttge 1973; Wieneke and Führ, 1973; Zimmermann *et al*., 1993).

Selvaraj (1998) demonstrated an expansion of photosynthetic action in leaves of *P. juliflora*, inoculated with *G. fasciculatum*. There was an improvement in chlorophyll content (chlorophyll a, b and total chlorophyll) in the leaves. Also, AM fungi appeared to increment stomatal conductance and photosynthesis after water stress in lemon (Levy and Krikun, 1970) and incremented both transpirational and photosynthetic rates just as chlorophyll amount in the grass *Bouteloua gracilis* (Allen, 1981). It was seen that bundle sheath chloroplasts were progressively high and that the veins and mesophyll cells of mycorrhizal finger millet were bigger than those of non-mycorrhizal plants (Krishna *et al*., 1981).

Roots colonized by AM fungi are thicker and convey less root hairs. Such changes in morphology are relied upon under phytohormonal control (Selvaraj, 1998). Abscisic acid (ABA) was observed to be significantly enhanced in the two roots and shoots of AM plants, when contrasted with non-mycorrhizal control (Danneberg *et al*., 1992). Indirect ELISA tests with polyclonal antibodies against ABA demonstrated that the degree of this phytohormone is twenty times higher in spores and hyphae than in roots of maize at all phases of plant growth (Danneberg *et al*., 1992). It is unlikely that ABA synthesized initially is by the maize cells, since AM organisms would then need to improve this phytohormone from the apoplasm, by active transport. It is increasingly likely that the organism applies control over the morphology of the roots and ABA assumes a significant job in this. ABA is associated with the regulation of soluble fluxes with in plants, which could likewise occur in AM fungi symbiosis (Druge and Schonbeck, 1992).

Additionally, enhanced IAA (Indole Acitic Acid), gibberellin and cytokinin level was seen in *G. fasciculatum* inoculated with *Prosopis juliflora*, as

recorded by Selvaraj (1998), who also demonstrated the impact of AM organisms, *G. fasciculatum*, on enhanced amount of growth hormones. Barea and Azcon-Aguilar (1982) likewise noticed that in axenic investigations, mycorrhizal organisms delivered auxin, gibberellin and cytokinin-like substances and enhanced plant growth.

CONCLUSION

The mycorrhizal plants had better nutrient uptake which was revealed by the content of phosphorous, nitrogen and zinc. Moreover, the tolerance towards drought stress is high in mycorrhizal plants than noonmycorrhizal plants.

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