J. Agric. Sci. Mansoura Univ., 30 (5): 2909 - 2919, 2005

EFFECT OF ARBUSCULAR MYCORRHIZA FUNGI ON GROWTH, TISSUE NUTRIENTS CONCENTRATION OF *Acacia saligna* AND ALKALINE PHOSPHATASE ACTIVITY IN THE RHIZOSPHERE

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ABSTRACT

The influence of arbuscular mycorrhiza fungi inoculation on growth performance of *Acacia saligna* (*A. saligna*) was studied in a greenhouse pot experiment at the Soil and Water Sciences Department, Faculty of Agriculture, Alexandria University. Soil samples were collected from El-Amal village, West Nubaria region for isolation of arbuscular mycorrhiza fungi. The arbuscular mycorrhiza genera found were *Acaulospora*, *Gigaspora* and *Glomus*, the amount of spores varied from 24-81 spores per 100 g of collected soils. The collected inocula were tested for their effectiveness in sporulation, it was found that *Glomus* produced the highest amount (5759 spores per 100 g soil). Inoculation with arbuscular mycorrhiza significantly improved the growth of *A. saligna*. Inoculation increased significantly plant biomass as compared with the uninoculated control plants. Inoculating *A. saligna* with arbuscular mycorrhiza fungi resulted in a 66.13, 53.33 and 85.46% colonization for *Acaulospora*, *Gigaspora* and *Glumos*, respectively. The difference between the root to shoot ratio of inoculated and uninoculated *A. saligna* was significant though the inoculated *A. saligna* had a higher root to shoot ratio as compared to uninoculated plants. Inoculation with arbuscular mycorrhiza increased significantly plant tissue nitrogen, phosphorus and potassium content. Protein percentage on *A. saligna* seedlings was high with *Glomus* (19.06%) and low with uninoculated treatment (12.43%). Also, alkaline phosphatase activity in the rhizosphere increased with inoculation with the three inocula and the concentrations of Fe, Zn, Mn and Cu were significantly increased by inoculation than uninoculation (control). Higher micronutrients concentration mean values of *A. saligna* were observed with inoculation by *Glomus*, being 450.16, 105.42, 185.21 and 10.23 μg g^{-1} dry weight for Fe, Zn, Mn and Cu, respectively.

Keywords: Arbuscular mycorrhyza, *Acacia saligna*, alkaline phosphatase activity, tissue nutrients content.

INTRODUCTION

The genus *Acacia* includes about 1200 species of trees and shrubs with a natural distribution in Australia, Asia, Africa and the Americas. Over 800 of these species are endemic to Australia. Few species are planted extensively outside their natural range for timber, pulpwood, tannin, fuelwood and erosion control. However, others are little known but have attributes that they could be more widely utilized to improve the well-being of people in developing countries.

Acacia "*Acacia saligna*" (synonym: *A. cyanophylla*) is a species of the genus *Acacia* that belongs to the plant family Leguminosae. It is a common tree being introduced along the north-western coast region of Egypt,

especially on sand dunes east and west Matrouh sectors (Aly and Hassan, 1993). It is used as windbreaks and for sand dune stabilization in Egyptian desert (Abd El-Rahman, 1967). It is characterized by tolerance to moderate ability to grow on poor soils, ability to bind sand, high production of biomass, high coppicing ability and high nutritive value for sheep and goat (El-Lakany, 1987). In a drip-irrigated plantation of *A. saligna*, established at the Experimental Station of the Desert Development Centre, the American University of Cairo, biomass production per tree averaged 6.5 kg of foliage and 7 kg of wood per annum during the first three years. *A. saligna* foliage was composed of 50-55% dry matter, 12-16% crude protein, 20-24% crude fiber, 6-9% crude fat and 10-12% ash (El-Lakany, 1987).

Mycorrhizas, symbiotic association between plant roots and beneficial soil fungi, are formed by the vast majority of woody plants and they function to enhance nutrient uptake particularly in infertile soils (Trappe, 1977). Many trees require mycorrhizas to survive and grow in natural forest ecosystems. The productivity of trees in plantations can be increased by inoculating seedlings in the nursery with selected mycorrhizal fungi (Garbaye and LeTacon, 1986).

Arbuscular mycorrhizal (AM) fungi are one of the most important biofertilizers, hence these fungi link plant and soil, transport nutrients to the plant roots and carbon compounds to the soil and its biota. Arbuscular mycorrhizal fungi may enhance plant growth by improving the supply of nutrients of low mobility in soil by direct and indirect modifications in the rhizosphere. The direct modification occurs by releasing hydrogen ions, chelating compounds or phosphatase enzymes (Bolen, 1991). Gianinazzi-Pearson and Gianinazzi (1978) stated that the maximum phosphatase activity occurred at the beginning of the infection (100% arbuscules) and declined thereafter with the development of both onion plants and the AM infection. They showed that the acid phosphatase was observed in the vacuolated, immature terminal arbuscules while the alkaline phosphatase was localized within the vacuoles of mature arbuscules and intercellular hyphae.

The objective of this study was to test the effect of arbuscular mycorrhiza inoculation on growth, tissue nutrients concentration, protein percentage and micronutrients concentration of *A. saligna* and activity of alkaline phosphatase in the rhizosphere of *A. saligna* seedlings.

MATERIALS AND METHODS

A greenhouse pot experiment was conducted at the Soil and Water Sciences Department, Faculty of Agriculture, Alexandria University. Surface soil sample (0-20 cm) was collected from El-Amal Village, West Nubaria region (46 km Alexandria-Cairo desert road and at 9 km east the road). The main chemical and physical properties of this soil were measured as outlined by Page *et al.*(1982) and the results obtained are given in Table (1). Soil was autoclaved for three successive days at 121°C for one hour daily and then distributed in black plastic pots at the rate of 6 kg pot^{-1}.

Nitrogen fertilizer as ammonium nitrate (33.5% N) was added at a rate equivalent to 30 kg N $fed.^{-1}$ and phosphorus fertilizer as mono-superphosphate (15.5% P_2O_5) at the rate of 20 kg P_2O_5 $fed.^{-1}$ was applied for all pots.

Table (1): The main chemical and physical characteristics of the used soil.

Characteristic	Mean value
pH*	8.2
EC* dS m^{-1}	2.1
Total $CaCO_3$ %	23.8
Organic matter %	0.1
Available macronutrients:	
Nitrogen mg kg^{-1}	49.2
Phosphorus mg kg^{-1}	4.3
Potassium mg kg^{-1}	116
Texture	Sandy loam

* Measured in soil water past extract.

Tree plant

Acacia saligna seeds were obtained from the Nursery of Forestry and Wood Technology, Agricultural Research Station, Faculty of Agriculture, Alexandria University at Abies, Alexandria.

Arbuscular mycorrhiza

Seventy four soil samples were collected from corn rhizosphere in El-Amal village, West Nubaria region. Mycorrhizal spores used in this study were originally extracted by a wet-sieving and decanting technique (Gerdmann and Nicolson, 1963) and sucrose centrifugation (Smith and Skipper, 1979).

One hundred grams of soil were suspended in one liter water by gentle stirring. Heavier particles were allowed to settle for a few seconds and the liquid was decanted through a 450 micron sieve to remove the large particles of organic matter and allow the spores to pass through. The suspension was passed again through 100 micron sieve. The spores and small size debris remained on 63 micron sieve were poured into centrifuge tube containing water, centrifuged at 2000 rpm. The upper solution was poured, the debris at the bottom was added with 40% sucrose and centrifuged for 2 minutes at 2000 rpm. The upper solution was separated and examined by the microscope. The spores which were collected and examined under the microscope were stored for identification.

Identification of arbuscular mycorrhiza

The identification of the obtained spores was carried out by following the monograph and the manual of Gerdmann and Trappe (1974) and Trappe and Schenck (1982) separate the spores into different genus by the spore attachment, size and colour. The classified spores were then multiplied in pot

cultures with onion. Three strains of AM were selected to test for their effectiveness on growth of *A. saligna*.

Seeds of *A. saligna* were immersed in sulphuric acid (99%) for 10 minutes then washed with distilled water several times until all traces of the acid were removed. The seeds were soaked in water for two days and germinated in mixture of 1:1 sand and vermiculite culture (ratio on volume basis) in greenhouse. Four weeks after germination, the plantlets were transplanted for one seedling per pot and five replicates were used for each treatment. For each AM fungus, inoculum consisted of a soil mixture containing heavily colonized roots of onion, spores and mycelium. Un-inoculated controls were included. The plants were irrigated when necessary with tap water to keep the soil moisture at 60% of their water holding capacity (26%) and were harvested 4 months after planting.

Sampling procedure

The plant roots were carefully washed from soil particles and randomized small pieces, about 1 cm length of fresh roots was used for AM infection percentage measurement using Giovannetti and Mosse (1980) method. Clearing and staining method of Phillips and Hayman (1970) with trypan blue stain was used for preparing root samples for microscopic observations.

Fresh and dry (oven-dried at 75°C for 48 hrs) weights of plants were recorded. Total nitrogen was measured by Kjeldahl method and protein percentage was calculated (Chapman and Pratt, 1961), phosphorus was determined by the vanadomolybdate yellow method and potassium was measured by flame photometry (Jakson, 1958).

Micronutrients (Fe, Cu, Zn and Mn) were also determined in the oven-dried plant materials according to methods outlined by Chapman and Pratt (1961).

Plants were collected carefully with root zone, then the rhizosphere soil samples were separated by gentle shaking for assay of alkaline phosphatase activity using Eivazi and Tabatabia method (1977).

The data were statistically analyzed using ANOVA procedure and the comparison means were tested using least significant differences analysis (SAS, 1990).

RESULTS AND DISCUSSION

Identification of arbuscular mycorrhiza and counting spores

Amounts of arbuscular mycorrhizal spores in collected and multiplied samples are shown in Table (2). Three genera of arbuscular mycorrhizal; *Acaulospora*, *Gigaspora* and *Glomus* were found. The highest amount of spores found was 81 spores/100 g soil and the least was 24 spores/100 g soil. The collected inocula were tested for their effectiveness in sporulation and infectivity. It was found as shown in Table (2) that *Glomus* produced the highest amount (5759 spores/100 g soil) while *Acaulospora* and *Gigaspora* produced lower amounts (2645 and 1600 spores/100 g soil, respectively).

Table (2): Amounts of arbuscular mycorrhizal spores in collected and multiplied samples.

Arbuscular mycorrhizal inocula	Description	Collected sample spores /100 g soil	Multiplied sample spores /100 g soil
Acaulospora	globose, hyaline, pale green, pitted-spore, surface 151x157 μ	45	2645
Gigaspora	globose, hyaline, white, pink, white, suspensor 233x236 μ	24	1600
Glomus	globose, subglobose, yellow brown, straight attachment 126x132 μ	81	5759

Inoculation and *Acacia saligna* growth

The fresh and dry weights of root and shoot of *A. saligna* seedlings inoculated with arbuscular mycorrhizal inocula are shown in Table (3). It is clear that inoculating *A. saligna* with arbuscular mycorrhiza significantly increased the fresh and dry weights of root and shoot. The response of *A. saligna* to mycorrhizal inoculation was found to depend upon the inoculum of arbuscular mycorrhiza used. The growth of seedlings was greatest in the *Glomus* inoculum and least in the *Acaulospora* inoculum. Inoculation increased significantly plant biomass as compared with the uninoculated control plants (Table 3). This observation was true for the three tested inocula.

Inoculation of *A. saligna* with *Glomus* inoculum increased root fresh and dry weights from 19.43 to 45.36 g plant^{-1} and from 3.75 to 4.64 g plant^{-1}, respectively. The percentage increase of these parameters were less in *Acaulospora* and *Gigaspora* inocula. Also, inoculating *A. saligna* with arbuscular mycorrhizal fungi increased significantly the shoot fresh and dry weights (Table 3). The shoot fresh weight increased from 33.29 to 51.80 g plant^{-1}, from 33.29 to 56.12 g plant^{-1} and from 33.29 to 61.82 g plant^{-1} with *Gigaspora*, *Acaulospora* and *Glomus* inocula, respectively. Also, the dry weight of *A. saligna* shoot was higher with *Glomus* (13.79 g plant^{-1}) than the other two inocula. At the end, the growth of inoculated *A. saligna* was highly significant as compared to the uninoculated plants.

The significant plant biomass production by the inoculated plants could be attributed to enhanced inorganic nutrition absorption and also to greater rates of photosynthesis in inoculated plants (Allen *et al.*, 1981).

Initial evidence of host specific colonization of *A. saligna* was provided by observation and examination of the morphological characteristics of the arbuscular mycorrhizal fungi. Root colonization of *A. saligna* seedlings showed abundant intraradical hyphae, variously shaped vesicles, some arbuscules and hyphal coils. Data in Table (3) show that the inoculating *A. saligna* with arbuscular mycorrhiza fungi resulted 66.13, 53.33 and 85.46% colonization for *Acaulospora*, *Gigaspora* and *Glomus*, respectively. There

was no arbuscular mycorrhiza contamination as evident in the uninoculated plants (control) which showed zero% colonization. It is obvious that mycorrhiza colonization is normally attributed to the tree species and environmental factors. Smith *et al.*(1979) reported that the extent to which typical vesicular arbuscular mycorrhiza fungi colonize root system varied with plant species.

Table (3): Fresh and dry weight of root and shoot and root colonization of *Acacia saligna* seedlings inoculated with arbuscular mycorrhizal inocula.

Arbuscular mycorrhizal inocula	Root weight (g plant^{-1})		Shoot weight (g plant^{-1})		Root colonization (%)
	Fresh	Dry	Fresh	Dry	
Uninoculated	19.43	3.75	33.29	10.73	-
Acaulospora	39.87	4.35	56.12	12.57	66.13
Gigaspora	31.17	4.22	51.80	11.81	53.33
Glomus	45.36	4.64	61.82	13.79	85.46
$L.S.D_{0.05}$	2.24	0.29	7.96	1.62	

Figure (1) shows the effect of inoculation with arbuscular mycorrhiza strains on root to shoot ratio of *A. saligna*. The difference between the root to shoot ratio of inoculated and uninoculated *A. saligna* was significant. The inoculated *A. saligna* had a higher root to shoot ratio as compared to uninoculated plants. The data indicate that the highest root to shoot ratio was obtained at inoculation with *Glomus* where these ratios were 0.58, 0.71, 0.60 and 0.73 for uninoculated, *Acaulospora*, *Gigaspora* and *Glomus*, respectively (Figure 1). It was reported that the higher root to shoot ratio of the inoculation plants could be attributed to the effect of mycorrhiza infection, which could increased nutrients absorption, giving rise to a higher root and shoot biomass increment (Clapperton and Reid, 1992). They also concluded that this high rate was due to that arbuscular mycorrhizal plants are being able to translocate more carbon to the roots than non-mycorrhiza plants.

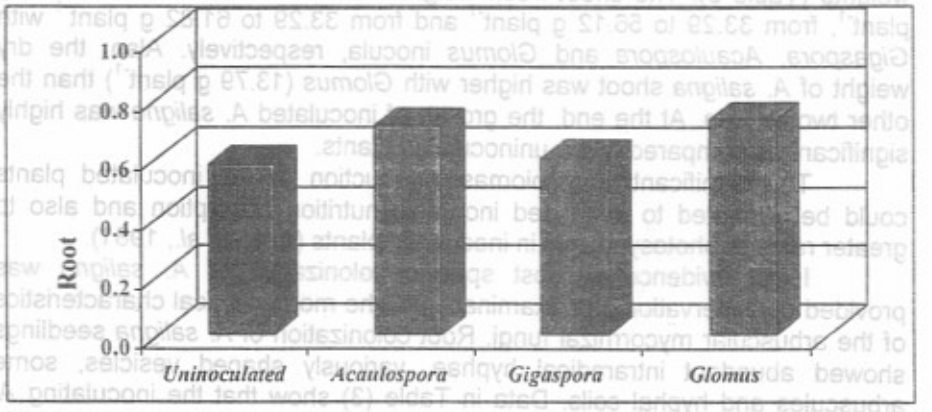

Figure (1): Effect of arbuscular mycorrhizal inocula on root/shoot ratio of inoculated *Acacia saligna* seedlings.

This point out that tree seedlings, with higher root to shoot ratio, are able to have a higher survival percentage when planted in the field.

Inoculation and plant tissue nutrients concentration

Table (4) shows that plant tissue nitrogen, phosphorus and potassium concentrations were much higher in the inoculated plants than in the uninoculated ones. Nitrogen, phosphorus and potassium concentrations were 1.99, 0.12 and 1.42% in the uninoculated plants, respectively. The seedlings inoculated with *Glomus* contained higher nutrient concentrations (3.05, 0.40 and 1.78% for nitrogen, phosphorus and potassium, respectively) than with *Acaulospora* or *Gigaspora* (Table 4). Ames *et al.*(1983) reported that about 24% of the total nitrogen uptake in mycorrhizal plants could be attributed to uptake and delivery by the external hyphae. This indicates that nitrogen is taken up by vesicular arbuscular mycorrhiza hyphae from inorganic sources of ammonium and, therefore, the higher nitrogen concentration in mycorrhizal plants could be attributed to the hyphae uptake. Michelsen and Rosendahl (1990) reported that mycorrhizal roots are able to absorb several times more phosphate than uninoculated roots from soils and from solutions. Mycorrhizal roots are known to have not only a considerably greater phosphate inflow rates, but also to possess a pathway of phosphate uptake with a much higher affinity for phosphate than non-mycorrhizal roots. This could also explain the higher potassium concentration in inoculated plants. Li *et al.*(1991) demonstrated that about 10% of the total potassium uptake in mycorrhizal coach grass was due to hyphal uptake and transport.

Table (4): Effect of arbuscular mycorrhizal inocula on nutrient concentrations in *Acacia saligna* plants.

Arbuscular mycorrhizal inocula	Nutrient concentrations (%)		
	Nitrogen	Phosphorus	Potassium
Uninoculated	1.99	0.12	1.42
Acaulospora	2.62	0.26	1.64
Gigaspora	2.25	0.18	1.53
Glomus	3.05	0.40	1.78
$L.S.D_{0.05}$	0.22	0.04	0.08

The response of *A. saligna* to mycorrhizal inoculation was found to depend upon the strain of arbuscular mycorrhiza used. Protein percentage of *A. saligna* seedlings was higher in the *Glomus* (19.06%) than in the uninoculated plant (12.43%). Inoculation increased significantly protein percentage as compared with the uninoculated control plants (Figure 2). El-Lakany (1987) reported that *A. saligna* is characterized by ability to grow on poor soils, high production of biomass, high coppicing ability and high nutritive value for sheep and goat.

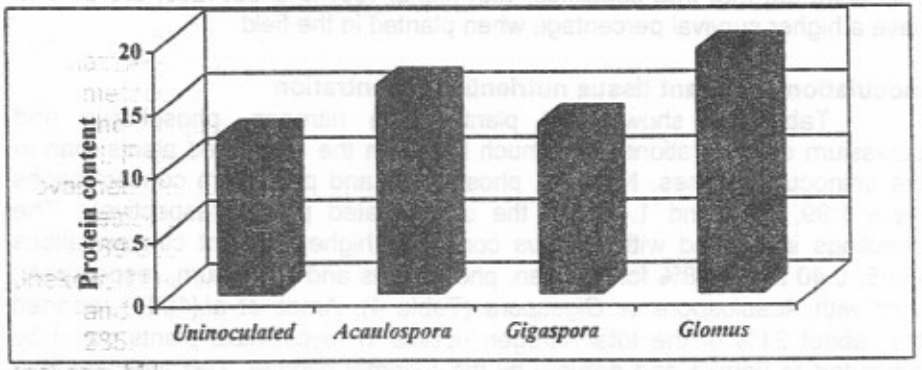

Figure (2): Effect of arbuscular mycorrhizal inocula on protein content (%) in inoculated *Acacia saligna* seedlings.

Inoculation and alkaline phosphatase activity

Data presented in Table (5) show the effect of arbuscular mycorrhiza on the activity of alkaline phosphatase in the rhizosphere of *A. saligna* plants. It is clear that alkaline phosphatase activity high significantly increased with inoculation with the three inocula. The alkaline phosphatase activity in uninoculated plants was 18.63 μg g^{-1} h^{-1} which is lower than that of *Gigaspora* was 42.28 μg g^{-1} h^{-1}. The soil rhizosphere of the inoculated plants with *Acaulospora* and *Glomus* had higher alkaline phosphatase activities. This indicates that mycorrhizal plants tended to increase the activity of alkaline phosphatase in mycorrhizosphere. This also may result by phosphatase excretion by mycorrhizal hyphae in mycorrhizosphere.

Table (5): Effect of arbuscular mycorrhizal inocula on alkaline phosphatase activity of inoculated *Acacia saligna* rhizosphere.

Arbuscular mycorrhizal inocula	Alkaline phosphatase activity (μg g^{-1} h^{-1})
Uninoculated	18.63
Acaulospora	50.19
Gigaspora	42.28
Glomus	65.40
$L.S.D_{0.05}$	4.56

Inoculation and micronutrients concentration in dry matter of plant

Table (6) indicated that the concentrations of Fe, Zn, Mn and Cu were significantly increased by inoculation than uninoculation (control). Results illustrated in Table (6) indicated that micronutrient concentrations in the nonmycorrhizal plants were 296.34, 37.29, 96.33 and 6.21 μg g^{-1} dry weight for Fe, Zn, Mn and Cu, respectively. Higher micronutrients concentration mean values were observed with inoculation by *Glomus*, being 450.16, 105.42, 185.21 and 10.23 μg g^{-1} dry weight for Fe, Zn, Mn and Cu, respectively. Inoculation with *Gigaspora* recorded the lower mean values of

micronutrients concentrations by *A. saligna* seedlings (Table 6). In this connection, Gnekow and Marschner (1989) reported that vesicular arbuscular mycorrhiza often enhances acquisition of phosphorus and micronutrients in plants.

Table (6): Effect of arbuscular mycorrhizal inocula on micronutrient concentrations (μg g^{-1} dry weight) of inoculated *Acacia saligna*.

Arbuscular mycorrhizal inocula	Fe	Zn	Mn	Cu
Uninoculated	296.34	37.29	96.33	6.21
Acaulospora	420.51	89.46	154.02	9.88
Gigaspora	327.11	71.27	120.52	6.93
Glomus	450.16	105.42	185.21	10.23
$L.S.D_{0.05}$	82.29	6.02	13.94	0.56

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تأثير التلقيح بفطريات الميكورهيزا الداخلية على نمو وتركيز العناصر الغذائية فى شتلات الأكاسيا ساليجنا ونشاط انزيم الفوسفاتيز القلوى فى الريزوسفير

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أصبح إستخدام التسميد البيولوجى ضرورة هامة لزيادة نمو النباتات المختلفة. وتعتبر الفطريات الجذرية (الميكورهيزا) من الأسمدة البيولوجية والتى لها دور هام فى زيادة صلاحية العناصر الغذائية للنباتات حيث أن هذه الفطريات متعددة العوائل. وقد أجريت تجربة أصص لدراسة تأثير التلقيح بفطريات الميكورهيزا الداخلية على نمو شتلات الأكاسيا ساليجنا وتيسر العناصر الغذائية الكبرى والصغرى والنشاط الإنزيمى للفوسفاتيز القلوى مستخدما عينات من الطبقة السطحية لأرض من قرية الأمل بمنطقة غرب النوبارية. وتم عزل جراثيم الفطريات الجذرية من تلك الأرض المستخدمة وتم إكثار الجراثيم المعزولة لاستخدامها فى تلقيح شتلات الأكاسيا ساليجنا.

كانت الجراثيم المعزولة تتبع الأجناس الثلاثة *Acaulospora*، *Gigaspora*، *Glomus* وبعد إكثار هذه الأجناس كان أفضل الأجناس هو *Glomus* حيث كان عدد الجراثيم ٥٧٥٩ لكل ١٠٠ جم أرض. أدى التلقيح بالميكورهيزا الداخلية إلى زيادة نمو الشتلات للأكاسيا ساليجنا زيادة معنوية مقارنة بالنباتات الغير ملقحة، وكانت إستجابة النباتات للتلقيح تعتمد على اللقاح المستخدم من الأجناس الثلاثة، وهذه الإستجابة كانت واضحة بالنسبة لأوزان الجذور والسيقان الرطبة والجافة للنباتات. وكان معدل إصابة جذور شتلات الأكاسيا ساليجنا بالفطريات الجذرية ٦٦,١٣، ٥٣,٣٣ و ٨٥,٤٦% للأجناس الثلاثة *Acaulospora*، *Gigaspora* و *Glomus* على الترتيب. كانت الزيادة فى نسبة الجذر إلى الساق بالنسبة للنباتات الملقحة مقارنة بالنباتات الغير ملقحة زيادة معنوية.

كما أوضحت النتائج أن محتوى النباتات من النتروجين والفوسفور والبوتاسيوم زاد زيادة معنوية نتيجة التلقيح بالفطريات الجذرية مقارنة بالنباتات الغير ملقحة حيث أن النباتات الملقحة بزيادة وتحسن نموها أدى لزيادة امتصاص هذه العناصر من الأرض. وكان نفس الاتجاه مع قيم نسبة البروتين حيث أعطى التلقيح بجنس *Glomus* قيمة مرتفعة لنسبة البروتين (١٩,٠٦%) عنها فى النباتات الغير ملقحة (١٢,٤٣%). وكذلك قيم النشاط الإنزيمى للفوسفاتيز القلوى أفضل فى حالة النباتات الملقحة، كما نتج عن التلقيح بالفطريات الجذرية زيادة تركيز العناصر الغذائية الصغرى فى النبات وكانت أفضل القيم مع التلقيح بجنس *Glomus* حيث كانت ٤٥٠,١٦، ١٠٥,٤٢، ١٨٥,٢١ و ١٠,٢٣ ميكروجرام لكل جرام مادة جافة لكل من الحديد والزنك والمنجنيز والنحاس على الترتيب.