Effect of vesicular - arbuscular mycorrhizal fungi on growth of olive seedings *(Olea europaea L.)* under different nutrient levels

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SUMMARY

The effect of vesicular-arbuscular mycorrhizal inoculation and fertilization level on olive growth (*Olea europaea* L.) was investigated. Olive seedlings were grown in a low phosphorus silt-clay soil and were either inoculated with *Glomus fasciculatum* Gerd. and Trappe and fertilized monthly with 0, 5, 10, 20 or 40g Crystalon per pot or non-inoculated without fertilizer application.

Mycorrhizal infection increased with the increase in levels of fertilizer application. Plant growth response to mycorrhizae was greater at low fertilizer level (5g/pot) and high rates of supplied fertilizer showed lower mycorrhizal effectivity. Shoot to root fresh weight ratio of mycorrhizal olive seedlings was the highest when grown at 10 and 20g fertilizer per pot. A positive correlation was found between leaf content of phosphorus and potassium and the level of supplied fertilizer. High levels of fertilizer appeared to inhibit the mycorrhizal efficiency and to stimulate non-mycorrhizal seedlings to grow as well as mycorrhizal seedlings. On the average, olive seedlings response to *G. fasciculatum* was the greatest at 5g nutrient regime.

Key words: *Glomus fasciculatum*, olive, growth, fertilizer, mycorrhiza.

INTRODUCTION

Endomycorrhizal fungi improves the nutrition and growth of most vascular plants (15). Growth stimulation of the host plant is generally attributed to increased uptake of phosphorus (P) by the relatively large and physiologically active root-fungus system (14). The relative host growth benefits, especially improved Plant P nutrition, may vary with soil P (8, 12), soil type (23), host cultivar (2, 22) and species of mycorrhizal fungus (21, 29). Mycorrhizal dependency is defined by (10) as the degree to which a plant is dependent on the mycorrhizal condition in order to produce its maximum growth or yield at a given level of soil fertility. A wide range of dependency in plants had been observed. Plant species are thought to differ in mycorrhizal dependency based on ability of non-mycorrhizal roots to absorb P from P defficient soils (12), (4) reported that species with long and abundant root hairs were less dependent than those with short or few root hairs. Among hardwood trees species studied, those with greater lateral root length and thinner root systems were less dependent on vesicular-arbuscular mycorrhizae (VAM) (29). Lateral roots generally

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have a greater incidence and intensity of infection than main roots (24). The mycorrhizal colonization of root system is influenced by host spp. and nutrition (7).

Olive roots have short and poorly distributed root hairs (17). The role of VAM in the nutrition and growth of olive plants has received limited attention. At present, the olive tree is receiving more attention in Jordan. The area with olive trees is increased from 16 to 30 thousand hectare from 1975 to 1985 (ministry of Agriculture, 1985). The objective of this work was to determine (i) the effect of VAM inoculation and fertilizer level on growth of olive seedlings and (ii) the effect of fertilization on VAM colonization of olive roots.

MATERIALS & METHODS

Four-months rooted cuttings of olive seedlings (*Olea europaea* L. cv. Nabali) were taken from the Faisal Nursery-Ministry of Agriculture. The seedlings were transplanted on Aug. 20, 1985 into 20-cm depth plastic pots containing an autoclaved silt-clay field soil immediately after mycorrhizal inoculation. The pH of the soil determined in 1:2.5 W/V water was 8.1 and each pot contained 5 Kg air dried soil. The soil contained 9 ppm of $NaHCO_3$ extractable phosphorus (25). Twenty grams of the infested soil as inoculum of the mycorrhizal fungus *Glomus fasciculatum* Gerd. & Trappe were added to the soil in each pot and mixed uniformely (100 spores/Kg of autoclaved soil). The fungus was isolated from Jordanian soils from olive trees in Al-Khaldeeh and classified according to Trappe (36). The inoculum consisted of soil, roots and spores from a pot containing *Zea mays* L. cv. Melogold which had grown for 90 days after being infected with *G. fasciculatum.* Non-inoculated seedlings received an inoculum water extract to establish the microflora associated with the inoculum in non-mycorrhizal treatments (11).

Growth of unfertilized non-mycorrhizal plants was compared with he growth of mycorrhizal plants at 0, 5, 10, 20 and 40g Crystalon per pot. Treatments were replicated six times and laid out in a randomized complete block design. Olive seedlings were fertilized after transplanting with a compound (Crystalon, N:P:K, 17:6:18) fertilizer dissolved in water. Each pot in different treatments received 0, 5, 10, 20 or 40g Crystalon every month. Plants were grown under glass-house conditions (18-36 C°) for 17 months. At harvest, fresh and dry weights of roots, tops and leaves were recorded. Leaves and roots of all plants in the same treatment were bulked and divided to three subsamples for all treatments except that with 40g Crystalon which was discarded because of early death of seedlings. Leaves and roots were oven dried for 18h at 80 °C and their phosphorus content was determined (25). Potassium content was determined in olive leaves (30). Percentage of host root colonization by *G. fasciculatum* was determined with a modified Phillips & Hayman (27) technique. The roots from each plot sample were washed free of soil, cut into one centimeter lengths and well mixed. From each bulk sample five grams were taken and prepared for microscopic examination by clearing the root system with 10% KOH, staining in lactophenol and trypan blue and examining 200 root segments mucroscopically. The percentage of root length which was mycorrhizal estimated visually under a research microscope and referred to as percentage of mycorrhizal colonization (5).

RESULTS

The means of shoot and leaves fresh weight of mycorrhizal olive seedlings at each nutrient level was significantly greater (P = 0.05) than the mean shoot and leaves fresh weight of non-mycorrhizal plants (Table 1). The mean fresh weight of mycorrhizal plants was small when 20g fertilizer was added. In this experiment, mycorrhizal olive seedlings grown at 5g fertilizer level were significantly larger in shoot fresh weight than mycorrhizal olive seedlings grown at 10 and 20g fertilizer levels. No significant differences were found in leaves fresh weight of mycorrhizal seedlings grown at 0 and 5g fertilizer regime but they were significantly greater in leaves fresh weight than mycorrhizal olive seedlings grown at 10 and 20g fertilizer levels. Leaves dry weight of mycorrhizal seedlings grown at 20g fertilizer level was significantly lower than mycorrhizal seedlings in other treatments (Table 1). Mycorrhizal seedlings grown under three fertilizer regimes contained higher percentage of dry matter than unfertilized non-mycorrhizal plants.

Table 1 – Effect of *Glommus fasciculatum* on shoot weight of olive seedlings under different fertilization regimes.

Mycorrhiza	Fertilizer g/pot	Shoot fresh weight g/plant	Leaves fresh g	Weight dry g
No *Glomus*	0	102.5 C*	39.2 C	16.7 C
Glomus	0	117.0 B	57.1 A	28.4 A
Glomus	5	132.1 A	62.8 A	28.7 A
Glomus	10	122.6 B	50.1 B	25.9 A
Glomus	20	117.1 B	46.8 B	20.9 B

*Means in a column followed by the same letter are not significantly different at 5% level according to D.M.R. Test.

Mycorrhizal olive seedlings showed a higher root weights than non-mycorrhizal plants only when plants grown under 0 and 5g fertilizer levels while roots of mycorrhizal plants grown under 10 and 20g fertilizer regimes were lower than non-inoculated ones (Table 2). No significant differences were found between roots of mycorrhizal plants either grown at 0 and 5g fertilizer level or at 10 and 20g fertilizer regimes. Mycorrhizal plants grown at all fertilizer regimes had higher shoot to root ratio than non-mycorrhizal olive seedlings. In this experiment mycorrhizal olive seedlings grown at 10 and 20g fertilizer levels had the highest fresh weight ratio of shoot to root (Table 2). No correlations were found between shoot fresh weight and shoot to root ratio.

Table 2 – Effect of *Glomus fasciculatum* on root weight and shoot/root of olive seedlings under different fertilization regimes.

Treatment mycorrhiza	Fertilizer g/pot	Root fresh weight g/plant	Root dry weight g/plant	Fresh weight shoot/root ratio
No *Glomus*	0	34.6 B*	14.9 C	2.96
Glomus	0	39.1 A	17.8 B	2.99
Glomus	5	41.3 A	20.9 A	3.20
Glomus	10	30.3 C	14.6 C	4.05
Glomus	20	28.6 C	14.1 C	4.09

*Means in a column followed by the same letter are not significantly different at 5% level according to D.M.R.Test.

The average content of phosphorus in olive leaves was the lowest (700 ppm) in non-mycorrhizal and highest in mycorrhizal olive seedlings (1840 ppm) grown under 20g fertilizer level. The content of phosphorus in leaf tissues of mycorrhizal plants grown under 0, 5, 10 and 20g fertilizer level were significantly different from control plants (Table 3). A positive correlation was observed between the phosphorus content and the amount of fertilizer. No correlations were observed between phosphorus concentrations in leaf tissues and shoot weight. Mycorrhizal olive roots contained higher P contents than in leaf tissues. A significant difference was observed between phosphorus contents of mycorrhizal roots and non-mycorrhizal. Adding high levels of fertilizer drastically increased P concentration in roots. Plants fertilized with 10 and 20g fertilizer showed no significant differences in their root's content of phosphorus. Significant differences were observed between mycorrhizal plants grown without fertilizer and others with higher rates of fertilizer. The mean potassium content in

Table 3 – Effect of *Glomus fasciculatum* on phosphorus and potassium content of olive seedlings under different fertilization regimes.

Treatment Mycorrhiza	Fertilizer g/spot	ppm of K in leaves	ppm of leaves	phosphorus in roots
No *Glomus*	0	8000 D*	700 E	900 D
Glomus	0	9050 C	1175 D	1325 C
Glomus	5	9200 C	1370 C	1575 B
Glomus	10	9550 B	1612 B	1825 A
Glomus	20	9900 A	1840 A	1912 A

*Means in a column followed by the same letter are not significantly different at 5% level according to D.M.R.Test.

leaves of the mycorrhizal plants exceeded than that of the plants without inoculation. In mycorrhizal plants, concentrations of potassium in leaves increased with increasing levels of fertilizer application. Concentrations of potassium were greater in leaves of mycorrhizal plants at the lowest fertilizer level than those in leaves from non-mycorrhizal plants (Table 3). No significant difference was observed between potassium contents in leaves of mycorrhizal plants fertilized with 0 or 5g fertilizer level. On the average, olive seedlings exhibited the greatest mean relative dependency on G. *fasciculatum* at the 5g nutrient regime and the least at 20g fertilizer level.

The percentage of root segments with G. *fasciculatum* structures and percentage of infected root length in roots from all treatments increasd significantly (P = 0.05) with increased fertilization. There was an average percentage of infected root segments of 33% at the 0 fertilizer regime and 53% at 20g fertilizer level (Table 4). Percentage of the root length colonized with G. *fasciculatum* was 15% and 23% at 0 and 20g fertilizer regime respectively.

Table 4 – Mycorrhizal infection of olive roots grown under different fertilizer regimes.

Treatment Mycorrhiza	Fertilizer g/spot	% of root segments with VAM structures	%of mycorrhizal colonization
No *Glomus*	0	0 C*	0 C
Glomus	0	33 B	15 B
Glomus	5	35 B	13 B
Glomus	10	40 B	18 AB
Glomus	20	53 A	23 A

*Means in a column followed by the same letter are not significantly different at 5% level according to D.M.R.Test.

DISCUSSION

These results illustrate some of the problems of comparing the efficiency of nutrient uptake into mycorrhizal and non-mycorrhizal plants grown in soil and under different nutrient regimes. Adding higher amounts of soluble fertilizer caused small reductions in dry weight of mycorrhizal roots (Table 2) which were accompanied by increase in shoot:root ratio. Such alterations in shoot:root equilibrium in response to application of nutrients have been well documented (6, 12, 16) and their interpretation depends greatly on measurements of rates of nutrient uptake based on the amount of absorbing tissue (root length or root weight) (35). In the experiments reported here, phosphorus and potassium contents in roots and shoots of mycorrhizal plants increased with the increased level of applied soluble fertilizer. High nutrient uptake was associated with reduction in root:shoot fresh weight ratio. The results as a whole provide a further example of the way in which nutrient absorption over the whole root

system may be controlled in relation to shoot growth, so that supplies of both mineral nutrients and photosynthate are optimal for plant growth. Mycorrhizal root systems were estimated to require 6 to 10% more photosynthate than non-mycorrhizal roots (18, 26). Phosphate uptake via mycorrhizas would be expected to affect this regulatory process. The results confirm that increased content in mycorrhizal plants from soils low in phosphate is associated with increase in shoot:root fresh weight ratio and a positive growth response to mycorrhizal infection as shown previously (31, 33, 34).

The ability of a plant to absorb P from low P soils is often thought to be the major contributing factor to mycorrhizal dependency (3, 8). Plants grown with less phosphate showed clear growth responses to infection (35) and high soil fertility substituted for mycorrhizal infection (19, 20). All mycorrhizal plants in these experiments had higher root and shoot concentrations of phosphorus and potassium (Table 3) than non-mycorhizal plants. Our results indicate that mycorrhizal infection contributed significantly to higher growth response at the lowest level of supplied fertilizer (5g/pot). Some reductions in percentage of infection of the root system by rapid growth of roots had occurred at low levels of fertilizer (Table 4), although percentage of infection of the root system was directly proportional to the amount of fertilizer supplied. These results emphasize the contribution made by growth of roots to percentage of infection, but is unlikely in our experiments, reduction in numbers of arbuscules sometimes occurs in soil high in phosphate (13). The percentage of root length infected was greater for seedlings receiving the low level of nutrient addition and the soil contained more spores than that receiving the high addition of nutrient (9). Abbott & Robson (1) have shown that adding phosphate did not affect numbers of arbuscules in *Trifolium subterraneum*. The results in this experiment confirmed the findings of Plenchette, Furlan and Fortin (28). Same, Robson & Abbott, (32) that high nutrient levels had inhibitory effects on mycorrhizal activity, since the high nutrient addition stimulated non-mycorrhizal seedlings to grow as well as mycorrhizal seedlings.

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تأثير الفطريات الداخلية (الأندومايكورايزا) على نمو شتلات الزيتون باستعمال عدة مستويات من السماد

مستخلص

تم دراسة التداخل بين الفطر المكون للأندومايكورايزا وكمية السماد وتأثير ذلك على نمو شتلات الزيتون. زرعت شتلات الزيتون في تربة طينية طميية ذات محتوى فوسفوري منخفض، ولقحت بفطر *Glomus fasciculatum* Gerd. & Trappe وأضيف السماد الثلاثي - كريستالون (18 - 6 - 17) للشتلات شهرياً وبمعدل صفر 5، 10، 20، و40 غم / نبات وتركت نباتات بدون معاملة كشاهد للمقارنة.

وجد أن تعايش الجذور مع فطر *G. fasciculatum* يزداد بزيادة كمية السماد المضافة بينما كان تجاوب النبات مع الفطر أعلى ما يمكن عند أقل كمية من السماد.

كانت نسبة وزن المجموع الخضري إلى المجموع الجذري أعلى ما يمكن في حالة إضافة 10 و20 غم/نبات، كما وجد أن هناك علاقة طردية بين محتوى الأوراق من الفوسفور والبوتاسيوم وكمية السماد المضافة.

إضافة كميات عالية من السماد قللت من فعالية فطر *G. fasciculatum* في تحسين نمو النبات وكانت النباتات لا تختلف إحصائياً عن الشاهد من حيث الوزن في حين أن إضافة 5 غم من السماد أعطت نمواً جيداً للشتلات المعاملة بالفطر.