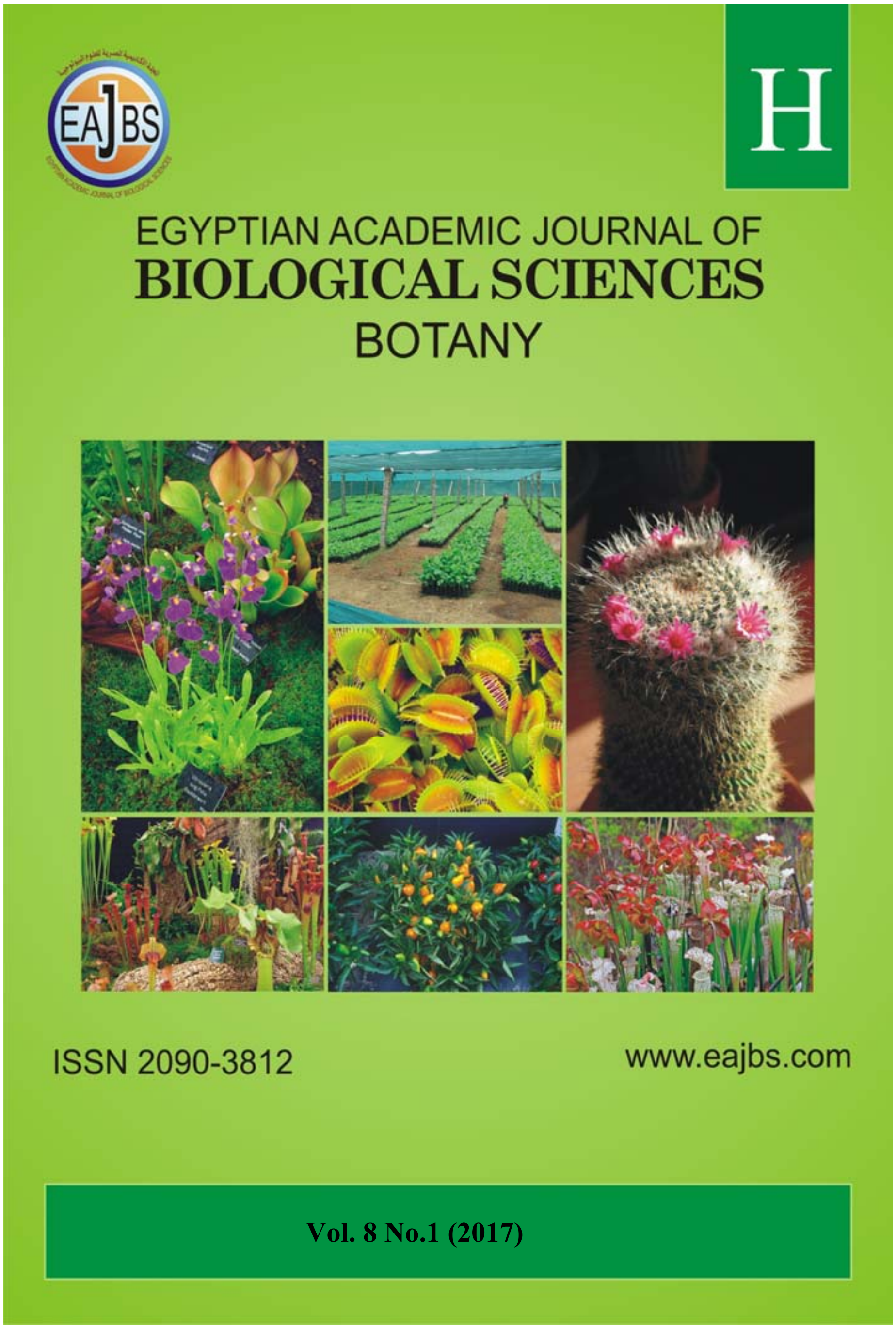

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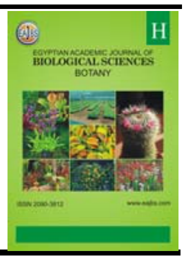

Effectiveness of three arbuscular mycorrhizae species on growth of some vegetable crops under calcareous soil conditions

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ABSTRACT

This work was aimed to examine the growth of some vegetable crops to select the species of AM fungi that can help to improve the growth of each vegetable crop especially under calcareous soil conditions and low P level. Surface calcareous soil sample (0-15cm) was collected from Abd El Baset village, Burg Al Arab, Alexandria - Egypt. Three levels of phosphorus fertilizer were applied before filling the pots to obtain P0 (without P fertilizer); P1 (50% of recommended P fertilizer (100 kg calcium superphosphate (15.5 % P_2O_5) / fed.)) and P2 (100% of recommended P fertilizer (200 kg calcium superphosphate (15.5 % P_2O_5) / fed.)). Three arbuscular mycorrhizal (AM) species were belonging to the genus *Glomus* used in this experiment. The applied species were *Glomus macrocarpium; Glomus intraradiaces* and *Glomus fasciculatum.* Three vegetable crops were examined, Summer Squash (*Cucurbita pepo* L.); Tomato (*Solanum lycopersicum*) and Carrot (*Daucus carota* L.). In conclusion, from the results of this study and under the same conditions of this experiment, we can recommended that, first, all mycorrhizal species were effective on plant growth and P content more than untreated plants, second the mycorrhizae specie *G. intraradiaces* developed the growth and P content of squash and tomato plants, while *G. macrocarpium* enhancing growth and P content of carrot plants under low P level and with calcareous soil conditions

INTRODUCTION

Vegetables are very important in keeping a healthy body. Some vegetables supply proteins, vitamins, oil and other essential nutrients which are necessary by the body to function normally (Gruda, 2005). Calcareous soils are those soils containing quantities of calcium carbonate that affect clearly the soil properties concerning to plant growth (FAO, 1984). The calcareous soils represent a wide area in the world and in Egypt; which about 12 million feddan. In general, calcareous soil is indigent in nutrients as nitrogen and phosphorus. Their low amount of nutrients is main reason of their low productivity (Shawky et al., 2004).

Phosphorus has been called "the key of life" because it is directly participatory in most life processes. It is a component of every living cell as a part of the nucleoproteins that load the genetic code of living things (Ozanne, 1980). It is also spirited in the energy transfer compounds with both plants and animal to carry on their life activities. Phosphorus is a limiting element for plant growth especially in low P soil of high Fe/Al or Ca contents, where P is hardly bound and largely unavailable for plant uptake (Marschner, 1995). Thus soluble phosphate fertilizer components once applied to the calcareous soil soon turn to less soluble or insoluble forms. Only 15 to 20% of the applied phosphorus fertilizer becomes available to the plants and a still smaller fraction to the succeeding plants (residual effect) (Egle *et al.,* 1999). The most real solution according to Marschner (1995), is the use of mycorrhizal fungi that have the ability to achieve P and produce high yield under limited P supply. Mycorrhizae are the most widespread association between microorganisms and plant roots (Abou El Seoud, 2005). Mycorrhizal fungi greatly increase the ability of plants to take up phosphorus and other nutrients those are comparatively immobile and exist in low concentration in the soil solution (Rouphael et al., 2010). The hyphae of the mycorrhizal fungus have the radius approximately 1.5 µm (Tinker *et al.*, 1992) and large surface area, leading to an increase of the root surface area which increasing of absorbing P, the production of organic acids and phosphatase enzyme which stimulate the release of P from organic components (Barea, et al., 2008). Enhancement of P uptake by mycorrhizal hyphae can also be indirectly due to the faster uptake rate of P by the AM hyphae and the disturbance of the solution solid P equilibrium, which will increase the uptake of absorbed phosphate into soil solution (Nye and Tinker, 1977). Mycorrhizae species differ in their ability to improve vegetable plants growth (Tawaraya *et al.*, 2012). A-mycorrhizae species having different responses to several vegetable crops nutrient uptake especially low mobile phosphorus (P), zinc (Zn) and cupper (Cu) (Pellegrino *et al.*, 2011). Effects of various mycorrhizal species on growth of vegetable crops were examined (Al-Hmoud and Al-Momany, 2017).

MATERIALS AND METHODS

This work was aimed to examine the growth of some vegetable plants to select the species of AM fungi that can help to develope the growth of each vegetable crop especially under calcareous soil conditions and low P level.

Soil preparation:

Surface calcareous soil sample (0-15cm) was collected from Abd El Baset village, Burg Al Arab, Alexandria - Egypt. The sample was air-dried, ground to pass into 2 mm sieve to homogenize and separate roots from soil, and thoroughly mixed before using. The chemical properties of the soil were as follows: pH (1:1 w/v water) 8.3, EC (1:1) 1.8 dS/m, organic matter 0.59%, $CaCO_3$ 21.05%, available nitrogen 113.3 mg/kg soil and available P 7.8 mg/kg soil (Olsen method). The soil properties were measured according to the methods described by Page *et al*. (1982)**.** Basal applications of N and K fertilizers were added to each kg soil at a rate of 150 mg N as NH_4NO_3 and 150 mg K as K_2SO_4 per kg soil. The N fertilizer was applied at three equal doses at the rate of 50 mg/20 ml water for each pot. The K fertilizer was applied before putting the soil in the pots. Three levels of phosphorus fertilizer were applied before filling the pots to obtain P0 (without P fertilizer); P1 (50% of recommended P fertilizer (100 kg calcium superphosphate (15.5 % P_2O_5) / fed.)) and P2 (100% of recommended P fertilizer (200 kg calcium superphosphate (15.5 % P_2O_5) / fed.).

Vegetable crops:

Summer Squash (*Cucurbita pepo* L.) were obtained from Nubaria Agricultural Research Station, the Ministry of Agriculture and Land Reclamation, Egypt. Tomato (*Solanum lycopersicum*) and Carrot (*Daucus carota* L.) were obtained from Central Laboratory for seeds Cairo, the Ministry of Agriculture and Land Reclamation, Egypt.

Arbuscular mycorrhizae:

Three arbuscular mycorrhizal (AM) species were belonging to the genus *Glomus* was used in this experiment. The applied species were *Glomus macrocarpium; Glomus intraradiaces* and *Glomus fasciculatum.* The first and third species were gained from Department of plant nutrition at Göttingen-University-Germany; the second specie was acquired from Department of plant pathology at Hanover University - Germany. All mycorrhizal species activated in the soil microbiology lab - Soil and Agriculture chemistry Dept. - Faculty of Agriculture - Saba Basha - Alexandria University-Alexandria, Egypt.

Pot experiment:

Plastic pots (12.5 cm in diameter and 11.5 cm deep) were washed, labeled, and a filter paper placed on the bottom of all pots to prevent soil seep, then a weight of 1 kg calcareous soil was placed for each pot leaving 5 cm upper without soil and compacted to bulk density of about 1.4 g cm^{-3}. One week before planting, all pots were watered to the volumetric moisture content 0.25 cm^3cm^{-3}, which correspond to the 70% of soil field capacity. Three seeds of vegetable plants (squash, tomato and carrot plants) were sown in each hill on March 15, 2016. The seedlings were thinned to one healthy and uniform plant per hill after 16 days from planting date. In mycorrhizal pots, the soil was mixed with 20 ml of mycorrhizae inoculums one week before planting (Malibari *et al.,* 1990). Also, 10 ml of mycorrhizal inoculums were added with the seeds at planting time, (in total, the rate of 500 A-mycorrhizal spores per pot). The treatments were replicated 5 times in completely randomized block design. All pots were irrigated with tap water every day to keep the soil at 70% of its field capacity by usual weighting of pots. The vegetable plants were harvested 57 days after planting (the harvest date May 11, 2016). At the harvest time, shoots of all vegetable plants were separated from roots. The shoots and half of roots (by weight) were washed with tap water, distillated water, air dried , and over dried at 70°C for 48 hours (Steyn, 1959) to constant weight and the shoot and roots dry weight were recorded, then grounded in a mill and stored in paper package for chemical analysis. Samples of plant material (shoots and roots) were wet digested withH_2SO_4–H_2O_2 (Lowther, 1980). Phosphorus content was determined by the vanadomolybdophosphoric method (Jackson, 1967).

Quantifying roots length:

Plant roots were separated from soil by washing them under a flow of tap water on a 0.5 mm sieve. Spare moisture was blotted from the selected roots by covering the roots in layers of paper towel for 2-3 min (Schenk and Barber, 1979). For each pot three roots samples of 0.3 g fresh weight were used for measuring the root length
by the line intersect method of Tannant (1975).

$$RL\ (cm) = \frac{RFW}{0.1g} \times N \times 1.5714$$

Where RL= root length, RFW = root fresh weight (g), N =sum of horizontal and vertical crossing.

Statistical methods:

The all data were arranged in a randomized complete block design (RCBD) and replicated five times. Data were statistically analyzed for ANOVA and means comparism to fulfill the significance according to Steel and Torrie (1982). The Tukey test was used to compare treatment means. A significance level of a = 0.05 was used in all analysis.

RESULTS AND DISCUSSION

The growth of some vegetable crops was examined to determine the response to several species of AM fungi under calcareous soil conditions.

1-Plant growth:

Shoot growth.

Phosphorus levels influenced the shoot dry weights of plants (Figure 1). Squash, tomato, and carrots without mycorrhizal fungi showed highly response to increasing P levels, indicating that plants without mycorrhizal fungi grew better at high P level than low P level. By other words, Based on shoot dry weight clearly differences were observed among the uninoculated vegetable crops at low and high P levels. Screening of squash, tomato and carrot for shoot dry weight may provide the best estimation on the productivity of the plant at low P soils. At high P level, the shoot dry weight of Squash, tomato, and carrots without mycorrhizal fungi increased by (4.19 fold; 2.55 fold; and 2.29 fold) respectively compared to the same plants at low P level. Whereas, the vegetable crops inoculated with mycorrhizal fungi were less response to increase P level. The supply of P fertilizer to the soil led to significantly enhanced uninoculated plant growth. However, when the soil was inoculated with mycorrhizal fungi, the effect of P fertilizer on plant growth remained less pronounced. This result is agreement with Neumann *et al.,* (2001) and Ortas *et al.,* (2001). The same results were observed for carrot (Ayalew, 2001 and Dechassa *et al.,* 2003), tomato (Oseni et al., 2010), and summer squash (El Kichaoui, 2016).

At high P levels, there was no significant difference between vegetable crops inoculated with arbuscular mycorrhizal species and the other plants without mycorrhizal inoculation. That could be due to AMF colonization reduced in soils with high soluble phosphate concentrations (Kaeppler et al., 2000). Increased the availability of P concentration in soil led to reduce mycorrhizal association, amount of spore production and genesis of secondary external AM hyphae (Lu *et al.,* 1994, and Valentine *et al.,* 2001). High phosphorus content in the tissue of plant root also decreased excretion of signal molecules that are responsible for AM hyphal branching and mycorrhizal association (Nagahashi and Douds, 2000). Moreover, cell phospholipids affecting on membrane permeability and emission of carbon compound that is necessary for mycorrhizal colonization (Schwab *et al.,* 1991). Muthukumar and Udaiyan (2000) reported that soluble carbon in cowpea root was increased with reducing tissue P levels. However, increased P concentration decreased extra-radical mycelium less than colonization (Olsson *et al.,* 1999). Factors decreased P soil solution affecting on mycorrhizal association and noticeably more mycorrhizal association was found in soils with high P fixing capacity (Lu et al., 1994).

At low P level, inoculation with different AMF species resulted highly significant increase in shoot dry weight of vegetable crops, but responded differently to the various AMF species (figure 1). The greatest growth (shoot dry weight) effects in squash plants were observed with *Glomus intraradiaces* (GI), and inoculation with the other two AMF species (GM and GF) led to higher shoot growth than that of the control (uninoculated plants) but significantly lower than the other squash plants inoculated with (GI) specie. While, the highly shoot dry weight of carrot were observed with *Glomus macrocarpium* (GM) followed with *Glomus intraradiaces* (GI) and there was no significant difference between them. On the other hand, there was no significant difference in shoot dry weight of tomato between the two mycorrhizal species *Glomus macrocarpium* (GM) and *Glomus intraradiaces* (GI) at low P level. Both (GM) and (GI) mycorrhizal species were significantly higher in tomato shoot dry weight than that the same plants inoculated with (GF) mycorrhizal specie or the uninoculated plants (control). Previous studies found that differences in vegetable growth depended on the species of AMF inoculant. These results are agreement with Kim *et al*., (2017). Similarly, Amer et al., (2010) studied the impact of AMF species on the growth of common bean and reported that *Glomus intraradiaces* was improved the plant growth than *Glomus* macrocarpium. Also, Baum et al., (2015) reported that, the effort of AMF to develop growth and quality of their host plants is controlled by the host specie, the AMF specie and their interactions.

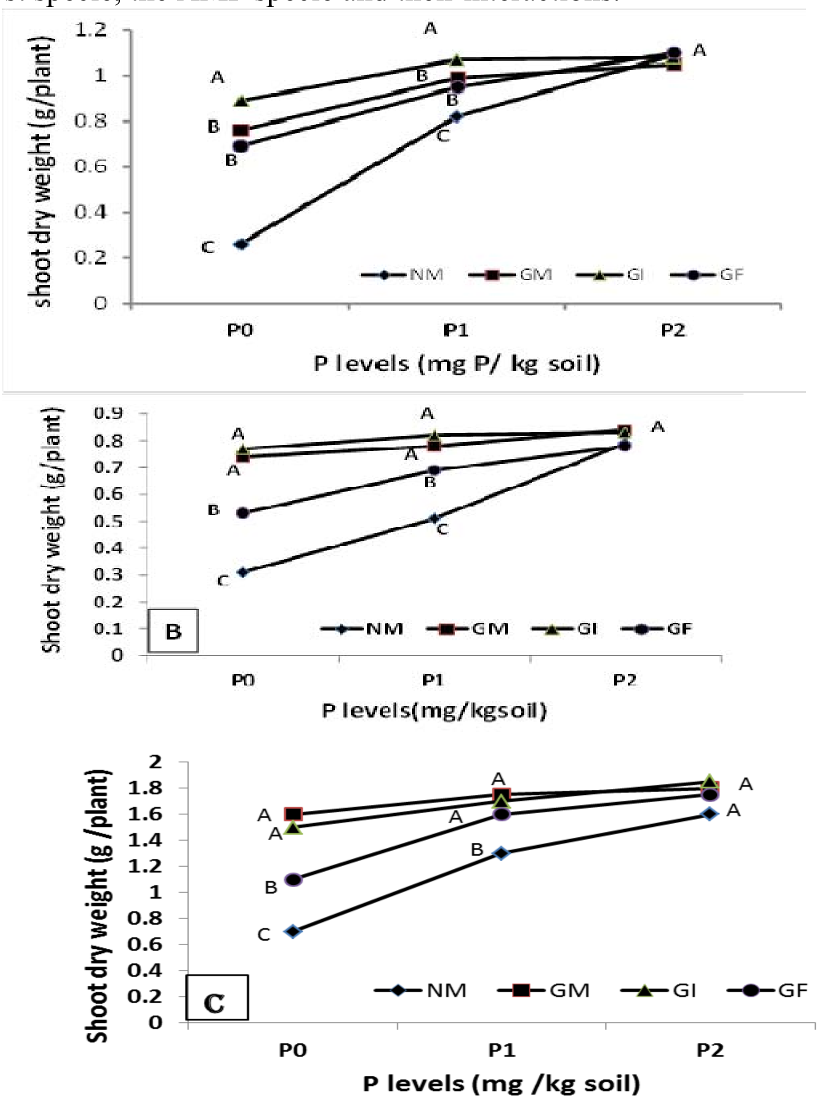

Fig. (1) : Shoot dry weight (g/ plant) of vegetable crops (A = Squash ; B = Tomato and C = Carrot) as affected by P levels and inoculated with mycorrhizal species; different letters indicate significant differences between mycorrhizal species at each P level, P≤0,05).

Root growth:

Plant having big root system can surrounds large volume of the soil and take up more P than those with small root system. Generally, a extensive root growth is the basis for keeping a high efficiency of nutrient acquisition, shoot growth and yield (Abou El Seoud, 2005).

As shown in (figure 2 and 3), there were highly significant increases in root growth parameters (root dry weight and root length) of different vegetable crops treated with A- mycorrhizal species compared with non-treated plants at low P level. In the same line, Neumann and George (2005) reported that, the root system of tomato plant was increased when treated plants with mycorrhizal fungi at low available P level. Also, mycorrhizal fungi may increase the root surface area of plant (SA) through increasing branches of first order lateral roots (Aguin *et al*., 2004), which due to increase uptake of nutrients by increasing the extension of soil depletion zone around the roots system. Plants having extensive root systems are able to absorb higher amounts of P from the soil and obtain greater yields than other plants having shorter root system (Abou El Seoud, 2008). Similarly, Amer and Abou-El Seoud (2008) found that the tomato root system inoculated with A-mycorrhizal fungi had increased significantly compared with untreated plants. Also, Alves *et al.,* (2001) showed that the adaptation of maize cultivars to low available P in soil is closely related to a better developed and extensive root system. In contrast, Bonanomi *et al*. (2001) reported that no significant difference in total root system was noticed when compared between plants inoculated with A-mycorrhizal fungi and other uninoculated plants.

Among the mycorrhizal species studied, *G. intraradiaces* led to improve root dry weight and root length of squash and tomato plants, while *G. macrocarpium* showed better root dry weight and root length of carrot plants under low P level compared with the other mycorrhizal species and other plants without mycorrhizal fungi (Figure 2 and 3). In the same line, Amer et al., (2010) reported that AMF specie *G. intraradiaces* was significantly increase the root system of tomato plants than the other AMF specie *G. macrocarpium*. Similarly, Drew et al., (2003) observed that plants inoculated with *G. intraradiaces* contained extensive root system than those plants treated with *G. mosseae.*

The root length of vegetable crops without mycorrhizae increased with increasing P levels. Similarly, Bloom *et al.,* (1993) reported that if the rhizosphere has low available nutrients, the growth of root system is slow. With development rhizosphere conditions, root system becomes more extensive. This result was agreement with Abou El Seoud (2005) who reported that, the root system density of uninoculated carrot (cm / cm^3) increased significantly with increasing the available P in soil. At high P level, there was no significant difference between the two mycorrhizal species *G. intraradiaces* and G. *macrocarpium* in root dry weight and root length of the three vegetable crops (squash, tomato and carrot).

On the other hand, the mycorrhizal specie *Glomus fasciculatum* was significantly lower than that the other two mycorrhizal species at all root growth parameters (root dry weight and root length) of the three vegetable crops (squash, tomato and carrot) as shown in (Figure 2 and 3) especially under low P level. In contrast, the root growth of plants inoculated with Glomus *fasciculatum* was significantly increase compared with the other vegetable plants without mycorrhizal inoculation.

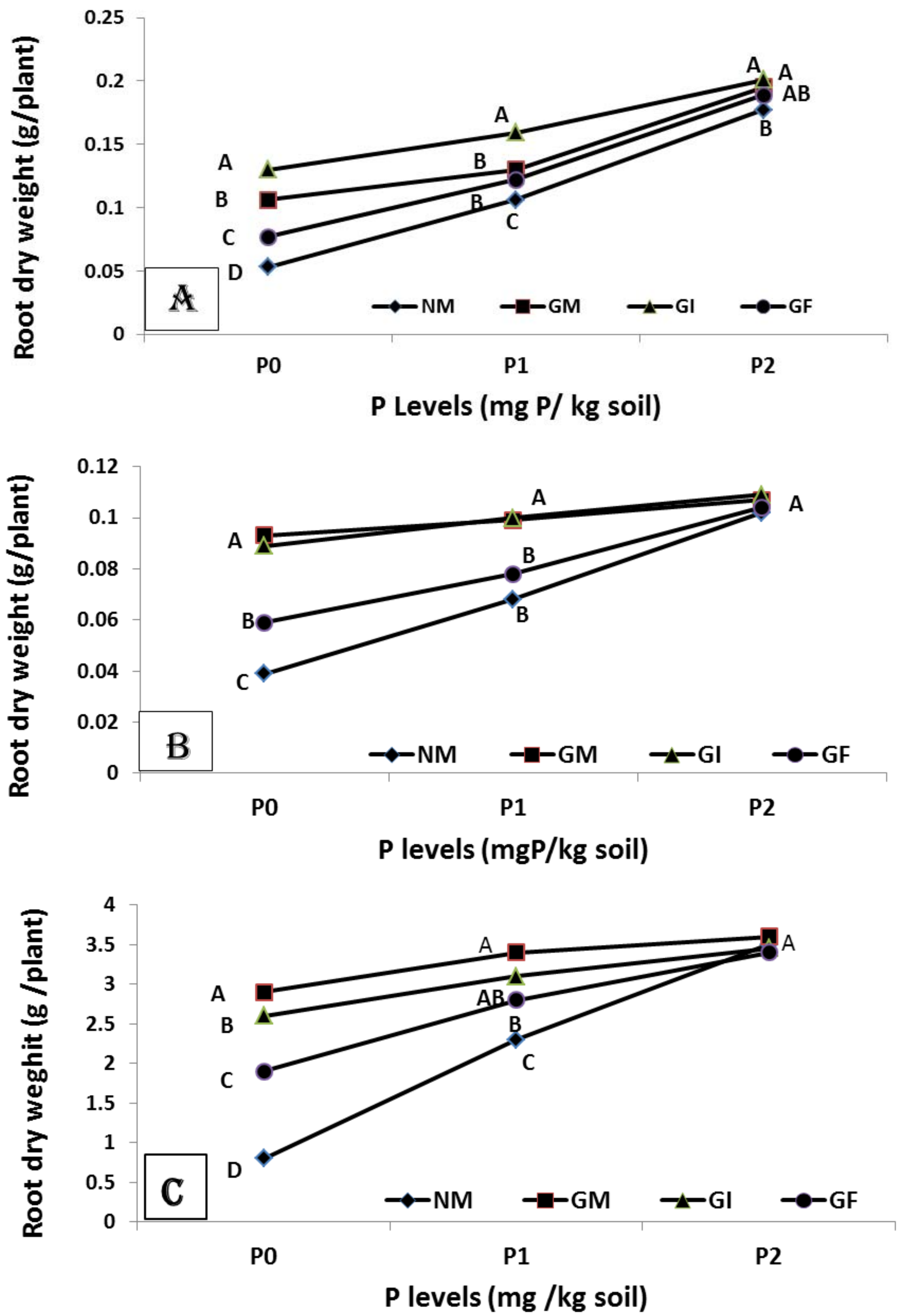

Fig. (2) : Root dry weight (g/ plant) of vegetable crops (A = Squash ; B = Tomato and C = Carrot) as affected by P levels and inoculated with mycorrhizal species; different letters indicate significant differences between mycorrhizal species at each P level, P≤0,05).

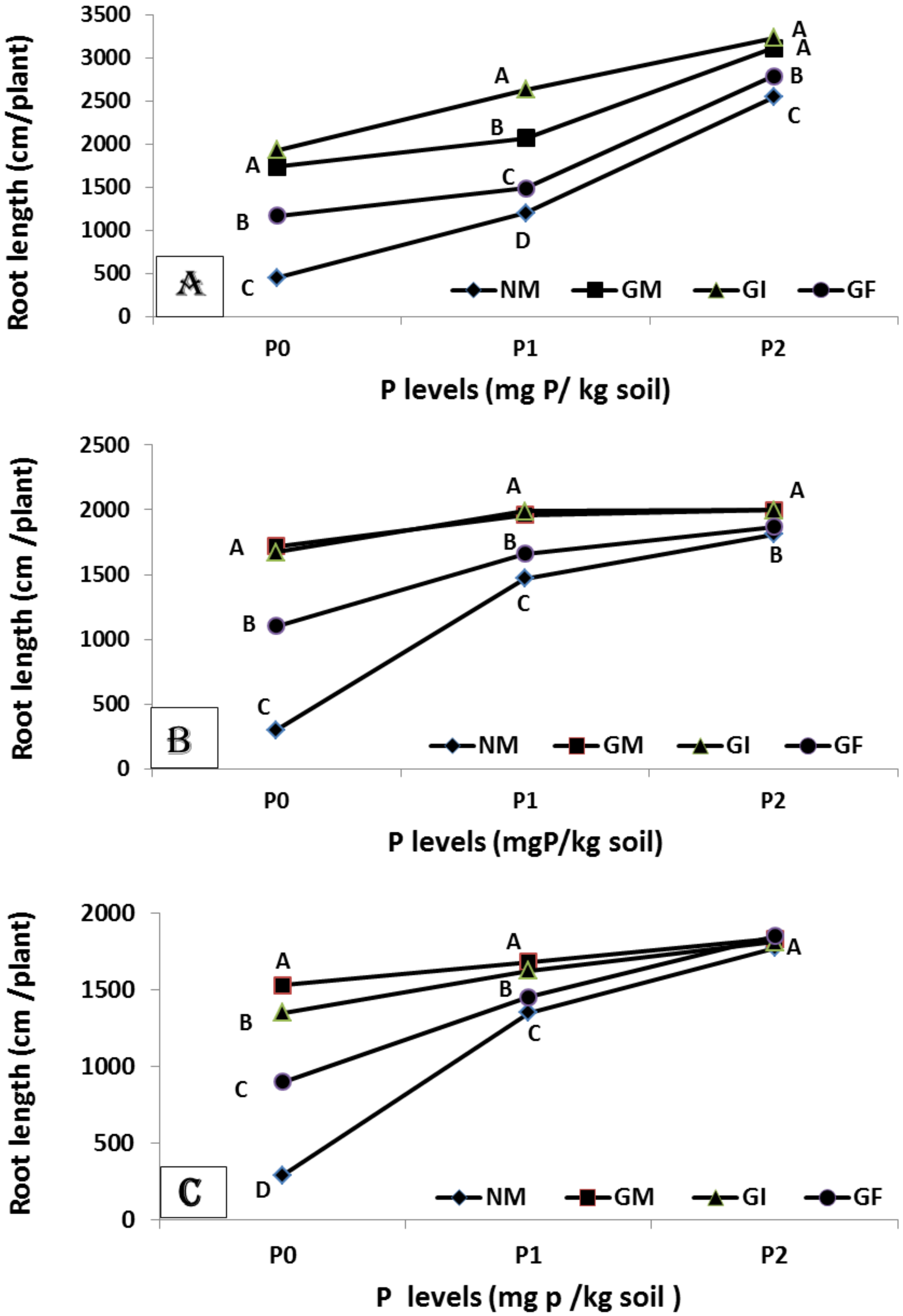

Fig. (3) : Root length (cm/ plant) of vegetable crops (A = Squash ; B = Tomato and C = Carrot) as affected by P levels and inoculated with mycorrhizal species; different letters indicate significant differences between mycorrhizal species at each P level, P≤0,05).

2-Plant phosphorus uptake:

The results clearly showed that, the shoot and root P uptake of all vegetable crops inoculated and uninoculated with AMF increased with increasing P levels (figure 4 and 5). The response of uninoculated vegetable plants to increase P level was higher than that the other plants inoculated with several mycorrhizal species. On the other hand, there was highly significant increase in shoot and root P uptake of all vegetable crops inoculated with all mycorrhizal species compared to the other plants without mycorrhizal inoculation (control) at the low level of phosphorus application (P0). These results are agreement with Gupta *et al.,* (2002) stated that inoculation plants with AMF significantly increased the uptake of P of mint plants which depleted the available P around the soil rhizosphere as compared to uninoculated plants. Also, Artursson *et al.,* (2006) demonstrated that, the plants treated with AMF significantly increased the accumulation of phosphorus (P) in plant tissues, compared with their control (uninoculated plants). In the same line, Meddad-Hamza *et al.,* (2010) reported that inoculated plants with A-mycorrhizal fungi had higher P and N contents than untreated plants. Similarly, Dennett et al., (2011) found that, treated of tomato plants with A-mycorrhizal fungi significantly improved root phosphorus content. That could be due to AMF develop the root surface area of plant by increasing branches of first order lateral roots (Aguim *et al.,* 2004), which due to increase uptake of nutrients (as N; P and K) by increasing the extension of soil depletion zone around the root system. An important effect of the AMF on plants growth is the reduction of phosphate deficiency (Tawaraya *et al.,* 2012 and Liu *et al.,* 2014).

In the same trend of shoot and root growth, among the mycorrhizal species studied, *G. intraradiaces* led to highly shoot and root P uptake of squash and tomato plants, while *G. macrocarpium* showed better shoot and root P uptake of carrot plants under low P level compared with the other mycorrhizal species and other plants without mycorrhizal fungi inoculation (Figure 4 and 5). Similarly, Heidari and Karami, (2014) studied the impacts of the two different mycorrhizae species *Glumus mossea* and *Glumus etanicatum* on grain yield, nutrient uptake and oil content of sunflower plants, and they reported that *Glumus etanicatum* had the highest impact on grain yield and nutrient content in seeds. In contrast, Akay et al., (2016) studied the impact of several species of arbuscular mycorrhizal (AM) fungi (*Glomus geosporum, Glomus mosseae, Glomus caledonium, Glomus etunicatium*) on plant nutrients content of lupin (*Lupinus albus* L.) and reported that there was no significant difference among the different species of A-mycorrhizal fungi treated to the lupin plant.

In conclusion, from the results of this study and under the same conditions of this experiment, we can have recommended that, first, it was concluded that all mycorrhizal species were effective on plant growth and P uptake more than uninoculated vegetable crops, second, the mycorrhizae specie *G. intraradiaces* developed the growth and P content of squash and tomato plants, while *G. macrocarpium* enhancing growth and P content of carrot plants under low P level and with calcareous soil conditions. That means, quality of host plants is controlled by the host plants species, A-mycorrhizal fungi species and their interactions.

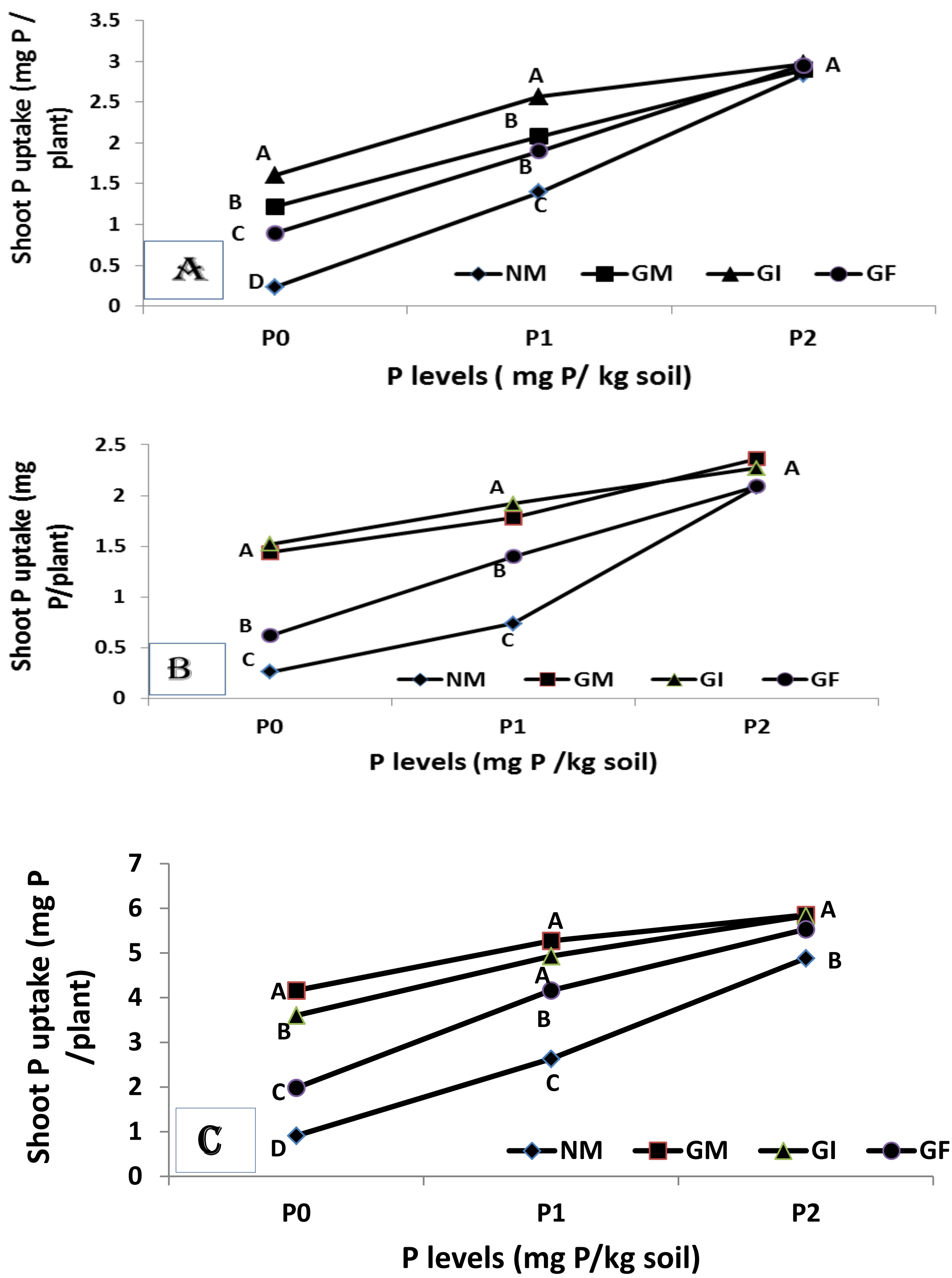

Fig. (4): Shoot P uptake (mg P/ plant) of vegetable crops (A = Squash; B = Tomato and C = Carrot) as affected by P levels and inoculated with mycorrhizal species; different letters indicate significant differences between mycorrhizal species at each P level, P≤0, 05).

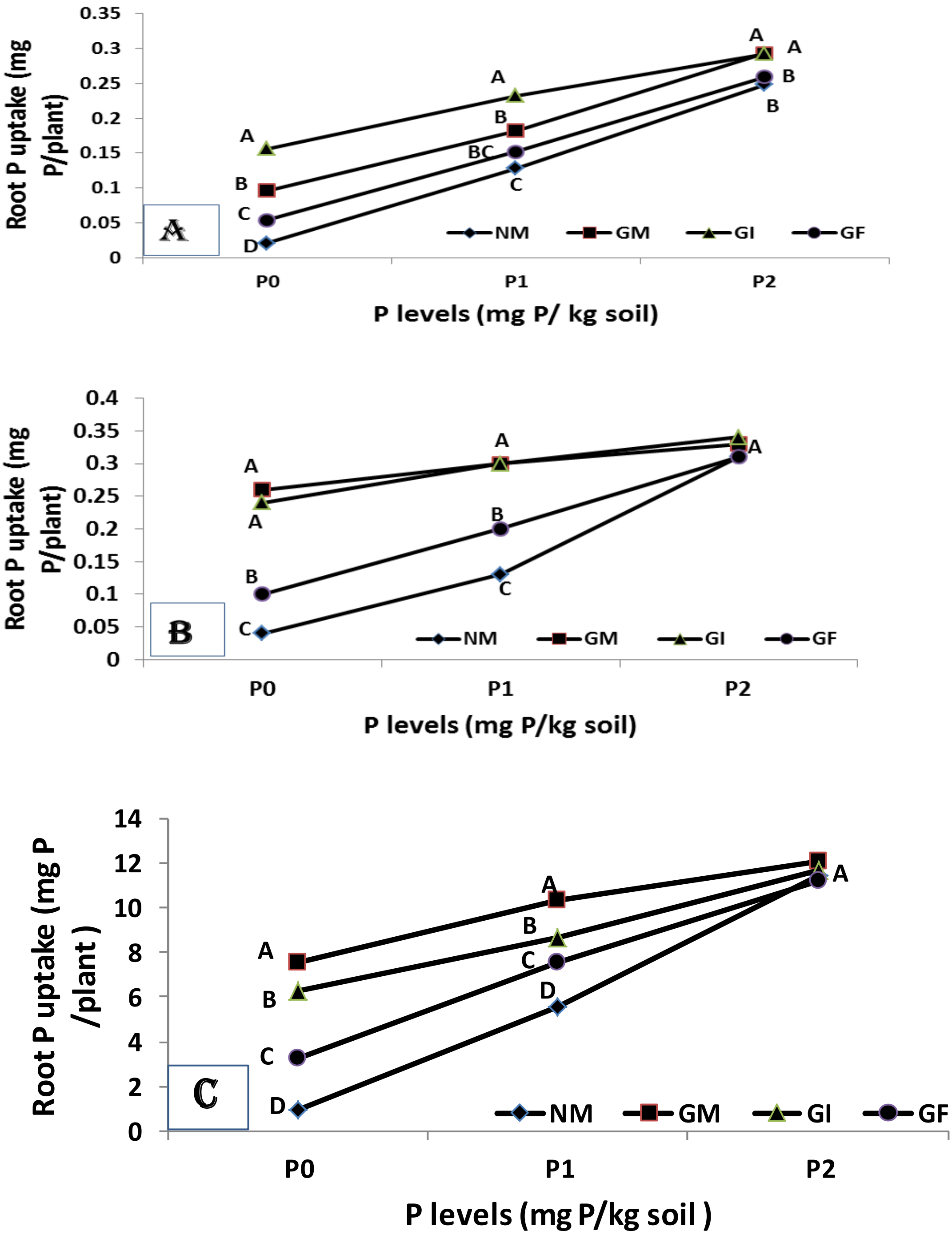

Fig. (5): Root P uptake (mg P/ plant) of vegetable crops (A = Squash; B = Tomato and C = Carrot) as affected by P levels and inoculated with mycorrhizal species; different letters indicate significant differences between mycorrhizal species at each P level, P≤0, 05).

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ARABIC SUMMERY

فاعلية ثلاث انواع من الميكوريزا على نمو بعض محاصيل الخضر
تحت ظروف الارض الجيرية

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يهدف هذا العمل الى اختبار نمو بعض محاصيل الخضر لتحديد انواع الميكوريزا التى تساعد على تحسين نمو أى محصول خضر وخصوصاً تحت ظروف الارض الجيرية ذات المحتوى المنخفض من الفوسفور . وقد تم اخذ عينة سطحية من التربة على عمق (صفر- ١٥سم) من قرية عبد الباسط – برج العرب – الاسكندرية- مصر . تم اضافه ثلاث مستويات من سوبر فوسفات الكالسيوم ١٥,٥ % P_2O_5 المستوى الاول: p_0 (بدون اضافة) المستوى الثانى p_1 (٥٠% من الجرعة الموصى بها) المستوى الثالث p_2 (١٠٠% من الجرعة الموصى بها) . إستخدم ثلاث انواع من الميكوريزا تنتمى الى جنس *Gloms* الانواع المستخدمة كانت *Gloms intraradiaces* and *Gloms macrocarpium* and *Gloms* fasciculatum وتم اختبار ثلاث انواع من محاصيل الخضر وهى الكوسة والطماطم والجزر ، فإنه تحت نفس ظروف هذه الدراسة نوصى بما يلى:

أولا : جميع أنواع الميكوريزا كانت مؤثرة على نمو النبات مقارنة بالنباتات التى لم تعامل

ثانيا : الميكوريزا من النوع *G.intraradiaces* قد أدى إلى تحسين نمو نباتات الكوسة والطماطم ومحتواهم من الفوسفور ، بينما نوع الميكوريزا *G .macrocarpium* قد أدى إلى زيادة فى نمو نبات الجزر ومحتواه من الفوسفور تحت ظروف الارض الجيرية ذات المحتوى المنخفض للفوسفور.