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# Mycorrhizae Can Support Squash Plant Growth in Phosphorus Deficient Calcareous Soil

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THIS WORK was aimed to select the proper Arbuscular Mycorrhizal (AM) inocula strain to enhance the growth of squash plants in calcareous P-deficient soil. Three treatments of phosphorus fertilizer of calcium triple phosphate, 15.5% $P_2O_5$ were tested at different rates. In parallel, pots were inoculated with 4 AM strains (*Rhizoglomus irregulare*) namely M49; M139; M301 and M 510. Treatments were repeated 5 times and arranged in the greenhouse in a randomized completely block design (RCBD) and plants were harvested after 57 days. In conclusion, from the results and under the same conditions of this experiment, we recommended that all mycorrhizal strains were effective in improving plant growth. The strain M301enhanced squash plant growth and P uptake. Also, inoculation with this strain could improve P availability in soil. The two mycorrhizal strains (M49 and M139) can be used for improving the growth of squash plants under low P level and in calcareous soil conditions. This study is one of the few studies that indicate the specialization of mycorrhizal fungi strains on the host plant and this study must be followed by many other studies in different climatic conditions, different soil properties and on different genus and strains of AM fungi on different plants family to confirm or deny this hypothesis.

**Keywords**: Squash plants, Mycorrhizal strains, P- fertilizer, Calcareous soil.

## Introduction

Squash (*Cucurbita pepo* L.) is known as one of the most important economic vegetables in the world due to its high nutritive amount being harvested in Egypt while other fruits are yet immature (FAO, 2009).

Calcareous soils represent a large area in the world especially in Egypt where about 12 million feddan were identified as calcareous. In general calcareous soil (with high $CaCO_3$ contents, alkalinity and buffer action) is relatively poor in nutrients specifically phosphorus. Its low nutrients content negatively affects productivity (Obreza and Morgan, 2008 and Abou Hussien et al., 2019).

Phosphorus (P) is the most effective nutrient in plant growth because it is the second main macronutrients after nitrogen (N) for crop growth (Heydari and Maleki, 2014). Phosphorous has an influential and pivotal role in energy conservation and transmission in the metabolism of the cell. P deficiency leads to stunting of seedlings and a decrease in plant root development. P-starved plants are usually stunted, appearance dark green and exhibit delayed plants developing as flowering and maturity.

Arbuscular Mycorrhizal (AM) fungi are found in the most soils of the world making an association with 80% of all economic plants (Harley and Harley 1987). The positive effects of AM fungi as a symbiotic association with the plants' growth were renowned (Smith and Smith 1996; Lakshman 2009 and Abdelhameid, 2020). The AM fungi extraradical hyphae can cross the nutrient depletion zone adjacent, to the plant root and thus improve the immobile elements availability as P, Zn and

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Cu (Elgharably and Allam, 2013) by translocating them from remote locations through the mycorrhizal hyphae to the plant roots, besides mobile elements as N (Grant et al., 2005). Treated some vegetables with AM fungi, may enhance plant growth performance (Temperini et al., 2009). The advantage of AM fungi and its inoculation depends on the combinations of genotypic host-fungus and also the type of the inocula which used (Rouphael et al. 2010). Kanwal et al., (2015) suggested that colonization of wheat plants with AM fungi improved biomass, growth and essential nutrients availability.

Few works are done to reveal the impact of AM fungi on *zucchini* growth and yield. Recent studies have demonstrated that treated *zucchini* with AM fungi could advantage (Colla et al., 2008 and Cardarelli et al., 2010) by improving its nutrition. Shehadeh (2010) reported that summer squash growth as roots and shoots is enhanced in the existence of mycorrhizal fungus spores suspension as compared to untreated plants. Furthermore, plant root growth increased significantly with mycorrhizal fungus spores suspension as compared with chemically treated plants. Inoculated plants with mycorrhizal fungi lead to increasing root growth to accommodate the mycorrhizal fungi mycelia accumulating inside. On the other hand, the effect of mycorrhization in plant shoot was clear and significant in activating the growth compared with the controlled growth. Similarly, Elkichaoui (2016) studied the impact of local endosymbiotic mycorrhizal fungus squash plants growth. Therefore, the study was begun by isolating the mycorrhizal fungus from squash seedlings roots cultured near an agricultural area. The results showed a positive effect of the mycorrhizal fungus on the growth of squash seedling comparing with control and other plants supplied with chemical fertilizers especially root growth systems. Also, Al-Hmoud and Al-Momany (2017) demonstrated that all mycorrhizal fungi strains had a positive effect on physiological squash plant content and its growth more than non-treated plants.

Few studies are known about the contribution of mycorrhizal fungi strains and their role on plants growth and nutrient uptake in calcareous soil. Abou El Seoud, (2008) studied P efficiency of tagetes plants treated with two strains of mycorrhizae (*Glomus intraradiaces,* M49 and M301). The hyphae length (HL) of mycorrhizal strain 49 was significantly longer than the HL of mycorrhizal strain M301 at a low level of P. Also, P uptake efficiency improved in the order M49 > M301 > NM fungi.

This work was aimed to select the proper Arbuscular Mycorrhizal (AM) inocula strain to enhance the growth of squash plants in calcareous P-deficient soil. Also, this study was aimed to demonstrate the specialization of mycorrhizal fungi strains on the different host plant.

## Materials and Methods

*Soil*

Soil was collected from Abd El-Baset village, Burg Al-Arab, Alexandria – Egypt, from the topsoil (thirty cm depth). The soil was air-dried, sieved through a 2 mm sieve and separate wheat roots from the soil, then mixed to homogenize before using. Soil chemical properties were as follows: pH (1:1 *w/v* water) 8.3, EC (1:1) 1.30 dS/m, organic matter 0.59%, $CaCO_3$ 21.05%, available nitrogen 113.3 mg/kg soil and available phosphorus 7.8 mg/kg soil (Olsen). The soil properties were determined according to the methods described by (Page *et al*., 1982).

*Plant materials*

Summer Squash (var. skata) (*Cucurbita pepo* L.) was getting from Nubaria Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt.

*Arbuscular mycorrhizae*

Four *Rhizoglomus irregulare* strains *i.e.*, M49, M139, M301 and M510 were obtained from Department of plant pathology at Hanover University, Germany and activated within the Soil Microbiology Lab, Department of Soil and Agriculture Chemistry, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt and treated as single-strain inocula in this work.

## Experimental Procedures

A greenhouse pot experiment was conducted at the Faculty of Agriculture (Saba Pasha), Alexandria University. Plastic pots (12.5 cm in diameter and 11.5 cm depth) were washed with distilled water, labelled, and a Whatman filter paper put in the bottom of each pot to prevent soil infiltration then a weight of 1 kg of tested calcareous soil was filled for each pot and leaving upper 5 cm without soil and compacted to bulk density of about 1.37 g $cm^{-3}$. Three squash seeds were sown in each pot and then seedlings were thinned to one healthy and uniform squash plant per pot 16 days after planting. Chemical fertilizers of N and K were applied for each kg of soil at the rate of 150 mg N as $NH_4NO_3$ and 150 mg K as $K_2SO_4$. The nitrogen fertilizer was added at three equal does at the rate of 50 mg/20 ml water for each tested pot. The potassium fertilizer was added before putting the soil into the pots. One-third of pots were fertilized with half of the recommended P-fertilizer (P1), i.e., 100 kg calcium triphosphate (15.5 % $P_2O_5$) / feddan, while one-third of pots were fertilized with the full dose of the recommended P-fertilizer (P2) i.e., 200 kg calcium superphosphate/ feddan. The last set of non-fertilized pots was included for comparison as a control (P0). In pots treated with mycorrhizal fungi strains, the soil was treated with 20 ml mycorrhizal spore suspension of the inocula strains (M49; M139; M301 and M 510) one week before planting as suggested by Malibari et al. (1990). Also, 10 ml aliquots of the inocula strains were added at planting time to reach a total spore density of 500 spores $pot^{-1}$. A set of pots without mycorrhizal fungi was used for comparison (uninoculated control). All treatments were replicated 5 times and arranged in a randomized completely block design (RCBD). Pots were regularly watered with tap water to reach 70% of the soil field capacity. Plants were harvested 57 days after planting. Shoots were separated from roots and weighed. The root system was separated into two parts by weight, the first part to measure root length and the second part for measuring P content. Shoots and half of the roots were dried at 70̊C in an oven for 48 hr (Steyn, 1959) to a constant weight which recorded then milled for measuring P content.

*P-analysis*

Plant samples were digested with $H_2SO_4$–$H_2O_2$ (Lowther, 1980). Plant phosphorus content was measured according to Jackson (1973). 100 g of air-dried soil samples from each pot were tested for phosphorus extraction at harvest time by sodium bicarbonate (0.5N) method according to Olsen et al. (1954) where the absorbance was spectrophotometrically at a wavelength of 406 nm (Murphy and Riley, 1962).

*Quantifying root length*

The root system of each zucchini plant has been separated from each pot by washing them under tap water on a 0.5 mm sieve. The excess moisture has been removed from these roots by wrapping them with layers of paper for 3 minutes to record a fixed total fresh root weight. (Schenk and Barber, 1979). Half of the root fresh weight per pot was collecting to measure root length. Three samples of 0.3 g root fresh weight were used for measuring root length by the line intersect method of Tennant (1975).

$$RL = (11/14) \times N \times G$$

where

RL = plant root length, N = sum of vertical and horizontal crossing.

G = the grid unit length (2 cm or 1 cm).

*Statistical analysis*

This experiment was arranged in a randomized complete block design with 5 replicates for each treatment. Data were statistically analyzed for ANOVA and means comparism to fulfil the significance according to Steel and Torrie (1982). Significanceat the 0.05 level was used in all analysis.

## Results

As illustrated in Fig. 1, shoot dry weight of non-mycorrhizal plants showed growth improvement in response to increasing P-fertilizer application levels, indicating that plants without mycorrhizal fungi can grow better with P-fertilizer application especially at a high rate. Shoot dry weight of the full P-dose plants was increased by about 5.23 folds without inoculation when compared to those received at low P-fertilizer rate. However, plants inoculated with mycorrhizae gained little improvement in shoot dry biomass due to increasing the rate of P fertilizer application. In other words, at high P level, the shoot dry weight of squash plants inoculated with the mycorrhizal strains M49; M139; M301 and M510 was only increased by about 1.49, 1.35, 1.17 and 1.65 folds, respectively compared with those fertilized with the low P level. Among all strains, the lowest response to increasing P-fertilizer application was exhibited by plants inoculated with strain M301 whereas, the mycorrhizal strain (M510) was the superior inocula strain concerning shoot biomass improvement. Squash plants inoculated with mycorrhizal strains M301 attained 85% of the shoot biomass yield already achieved by plants grown at without P-fertilization. In contrast, at the same level of available P, plants without mycorrhizae attained as low as 19% of their highest yield. For plants fertilized with the full-P- fertilizer dose ($P_2$), there were no significant differences in shoot dry weights between squash plants inoculated with the different strains and those without inoculation as illustrated in (Fig. 1). However, in the non-fertilized pots (P0), inoculation with all strains resulted in statistically significant increases in shoot dry weight. The maximum shoot dry weight was recorded with plants inoculated with strain M301, followed by those inoculated with strains M49 and M139 which led to significantly higher shoot growth than the mycorrhizal strain (M510) and the uninoculated control plants but significantly lower than those inoculated with strain M301. On the other hand, there was a significant difference in shoot growth of squash plants between plants grown with the mycorrhizal strain (M510) and the control plants.

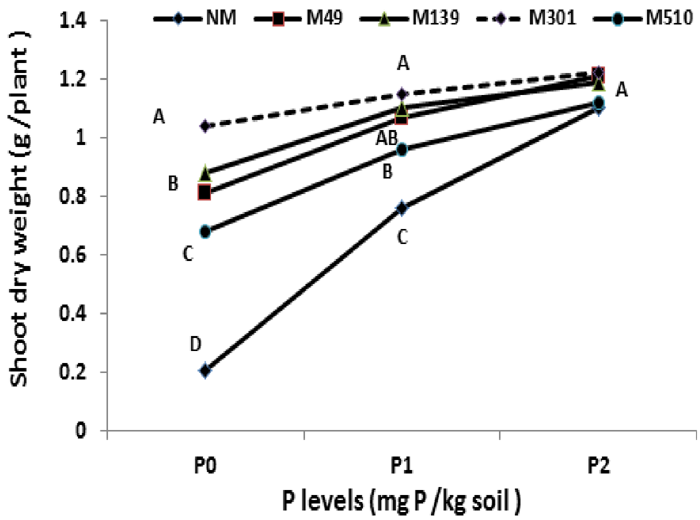


**Fig. 1. Shoot dry weight (g/ plant) of Squash as affected by P levels and inoculation with different mycorrhizal strains; different letters indicate significant differences between mycorrhizal strains at different P level, P≤0.05**

The effect of mycorrhizal inoculation was more pronounced in pots grown without P-fertilizer application (P0) with statistically significant differences recorded in root growth parameters between squash plants grown with mycorrhizal strains and uninoculated ones. The root lengths of mycorrhizal squash plants inoculated with strains M301, M49, M139 and M510 were around 7.36, 4.70, 5.19 and 2.67 folds higher than the uninoculated controls, respectively, (Fig. 3). The lowest root length and dry weight of squash were recorded with uninoculated plants at the lowest P level. However, improvements in both root growth parameters could be obtained as a result of the dose of P-fertilizer application. Inoculation with mycorrhizal strains improved both root dry weight and root length particularly in non-fertilized pots ($P_0$) and those supplied with the half P-fertilizer dose ($P_1$) (Fig. 2 and 3). Half dose P-fertilizer application ($P_1$), resulted in statistically significant differences in root dry weight between mycorrhizal and non-mycorrhizal plants except M510. Also, for plants grown with the full dose of P-fertilizer ($P_2$), a significant difference was recorded in root length between plants inoculated with mycorrhizal strains and uninoculated controls.

Figures 4 and 5 show increased shoot and root P uptake in both mycorrhizal and non-mycorrhizal squash plants due to increasing P-fertilizer application rate. This was more pronounced in uninoculated squash plants than inoculated ones. Statistically significant increases were recorded in shoot and root P uptake of squash inoculated with all mycorrhizal strains when compared with controls in pots received no-P- fertilizer ($P_0$) or those supplied with half P-fertilizer dose ($P_1$). In comparison with uninoculated controls, the P uptake of squash plants (shoot and root) treated with mycorrhizal strains M49; M139; M301 and M510 was magnified about 10.9, 10.8, 17.18 and 6.91 folds at no P fertilized soil. Nevertheless, inoculation with mycorrhizae did not have a significant impact on P uptake of squash plants fertilized with the full P-fertilizer dose ($P_2$). The mycorrhizal strain M301was the superior inocula strain in stimulating plant P-accumulation in shoot and root.

As illustrated in Fig. 6, the available P in the soil of all plants with and without mycorrhizal inoculation increased significantly as a result of increasing P applications. The response of untreated plants to increase P levels was significantly higher than the other treated plants. Bu other words, at (P0), there was a significant difference between plants treated with AM fungi and the untreated plants. In contrast, at high P level (P2), there was no significant difference in available P in soil between plants with and without mycorrhizal inoculation. On the other hand, at the first two P levels (P0 and P1), the plants treated with mycorrhizal strain M301 observed significantly highest available P in the soil as compared with other mycorrhizal strains and uninoculated plants. The soil available P content was almost not affected by the inocula strain application in pots received the full dose of P-fertilization. Meanwhile, the positive effect of mycorrhizal inocula strains was more obvious in pots received at no P-fertilizer ($P_0$).

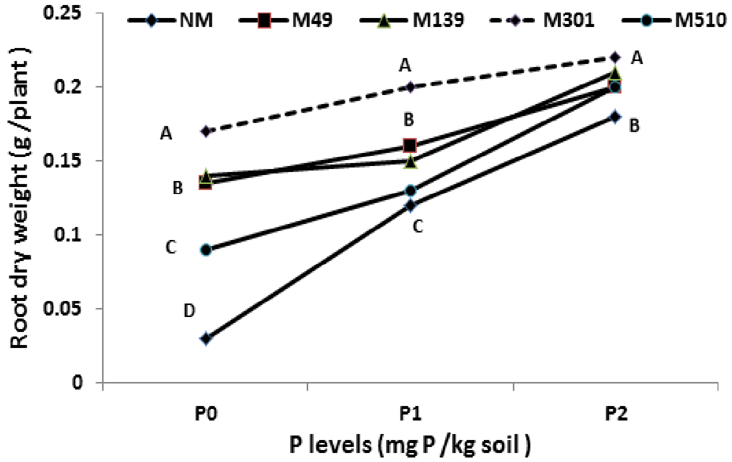


**Fig. 2. Root dry weight (g/ plant) of Squash as affected by P levels and inoculation with several mycorrhizal strains; different letters indicate significant differences between mycorrhizal strains at different P level, P≤0.05**

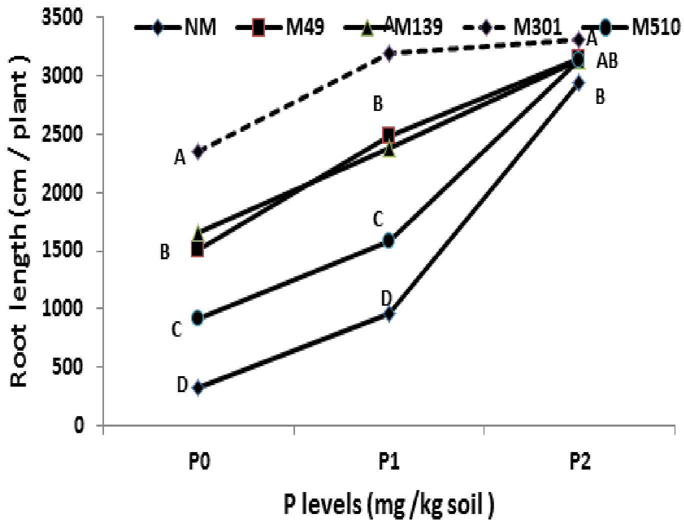


**Fig. 3. Root length (cm/ plant) of Squash as affected by P levels and inoculation with several mycorrhizal strains; different letters indicate significant differences between mycorrhizal strains at different P level, P≤0.05**

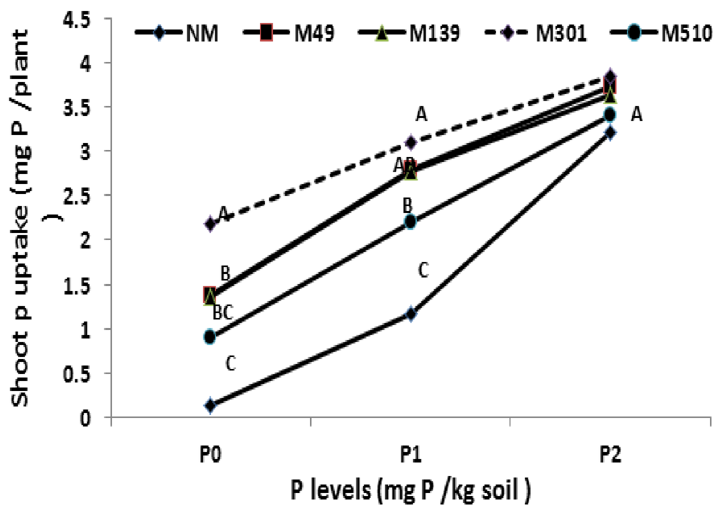


**Fig. 4. Shoot P uptake (mg P/ plant) of Squash as affected by P levels and inoculation with several mycorrhizal strains; different letters indicate significant differences between mycorrhizal strains at different P level, P≤0.05**

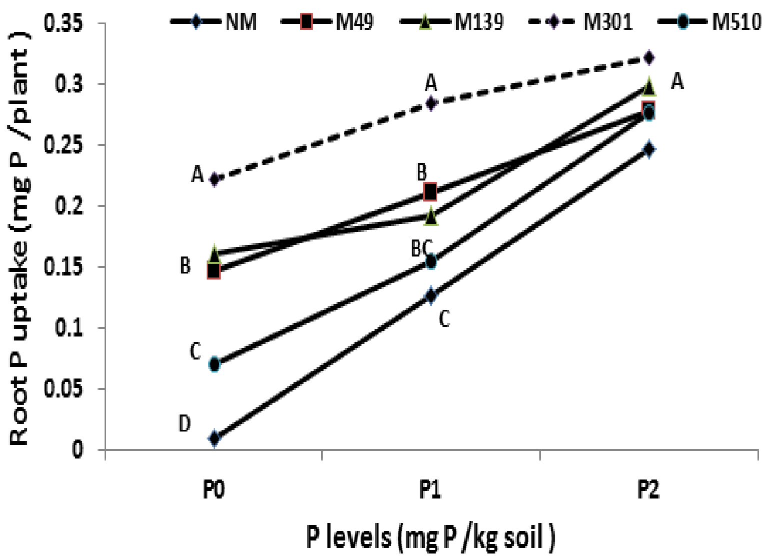


**Fig. 5. Root P uptake (mg P/ plant) of Squash as affected by P levels and inoculation with several mycorrhizal strains; different letters indicate significant differences between mycorrhizal strains at different P level, P≤0.05**

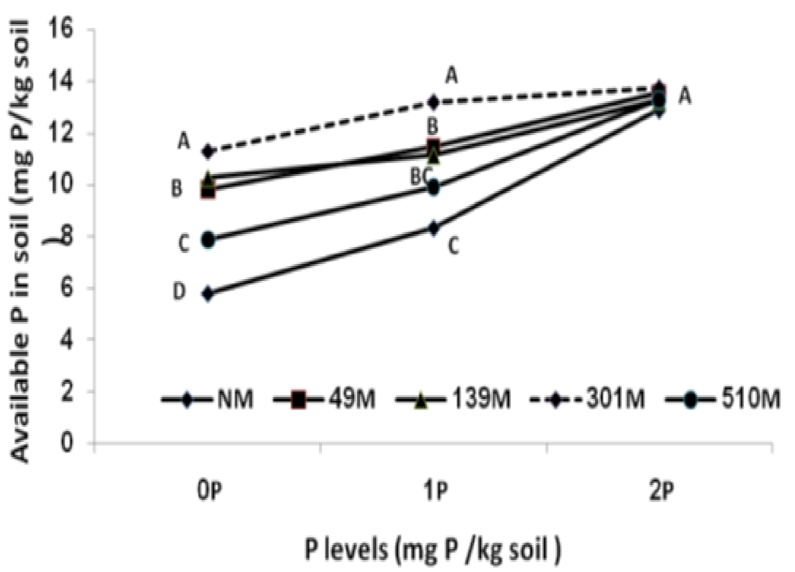


**Fig. 6. Available P in soil (mg P/ kg soil) of Squash as affected by P levels and inoculation with several mycorrhizal strains; different letters indicate significant differences between mycorrhizal strains at different P level, P≤0.05**

## Discussion

The obtained results refer to improved growth of non-mycorrhizal squash plants as a result of P-fertilizer application. Similarly, Alt and Ladebusch (1984) reported that the yield of plantsenhanced significantly with increasing P levels. Also, Abou El-Seoud (1998) demonstrated that the cotton shoot yield without mycorrhizal fungi was improved significantly with increasing P supply. In the same line, Dechassa et al. (2003) reported that the reaction of carrot to P supply was vigorous. Also, Abou El Seoud et al. (2017) observed that increasing phosphorus fertilizer levelsimproved the shoot dry matter of squash, tomato, and carrots plants without AM fungi inoculation, which means untreated plants with AM fungi grew better at high P rate as compared with low P rate. Phosphorus efficiency can be mostlyknown as the capability of a plant to produce a maximum yield withlow amount of phosphorus supply in soil (or other media) (Gourley et al., 1994). Similarly, could be known as the capability of a crop plant to produce a certain percentage of the maximum crop yield that can be achieved (80% of maximum yield) at the low amountP level in soil (Föhse et al., 1988). This signified that squash plants treated with the AM strain (M301) which obtained 85% of maximum shoot dry weight at low P level had high P efficiency, whilst squash without mycorrhizal fungi inoculation had low P efficiency. That could be affected by plant root system and/or hyphae length of AM fungi. Then squash treated with AM fungi strain (M301) was tolerant to low available P in soil as compared to squash without AM fungi inoculation. This result is in agreement withCavagnaro et al. (2003) who suggested that, inoculated plants by A-mycorrhizal fungi developedthe plant growth at low available P. Also, Abou El Seoud (2008) found that tagetes plants which treated with AM fungi strains achieved more than 80% of its maximum yield at the lower level of P in soil. Similarly, Abou El Seoud (2005) demonstrated that, shoot yield of carrot treated with A- mycorrhizal strain (namely, M49)was increased significantly compared to other carrot plants without mycorrhizae inoculation. That may be due to the function of mycorrhizal fungi in developing the plant root system (Abou El Seoud et al. 2018); producing mycorrhizal hyphae length with a fine radius which increased depletion of the nutrients from the soil especially P (Abou El Seoud, 2019). The power of mycorrhizal fungi hyphae can extend into the soil far behind the root system surface and/or root-hair zone. Also, their fineradius can extend into soil pores that roots with much larger diameters are unable to access in it. This means that they can access solution contains soluble nutrients filled soil pores at extremely lower soil water potentials than roots and hence can absorb soluble nutrients as P from drier soils (Jakobsen et al., 2005).Abou El Seoud et al. (2020) suggested that plant root system and mycorrhizal hyphae length is the main factors suitable for selecting P-efficient wheat genotypes, especially under limited Psupplies. Also, secretion of acid phosphatase into the soil from the external A- mycorrhizal hyphae is improved under low level of P conditions (Ezawa and Saito 2018 and Sato et al., 2019).

Mycorrhizal fungi improved squash shoot growth. This impact was more obvious at low P level in soil but not at high P supply. The effect of chemical fertilizer application usually reduces the density of root colonization; AMF spores and hyphae length (Sato et al., 2019) but only a few studies have demonstrated their long-term impacts on AM fungi extraradical hyphae development (Gryndler et al., 2006 and Wilson et al., 2009). Kahiluoto et al. (2001) observed reduces in AMF colonization densities under various soil and climate conditions upon supplies of greater than 50 kg P $ha^{-1}$. Also, Ryan and Graham (2002) reported that high amount of available P in soil often limits A- mycorrhizal fungi colonization, inhibit both spore germination and early mycorrhizal hyphae growth (Miranda and Harris, 1994). That may be due toimprovingthe levels of phospholipids, which reduce membrane permeability and decrease exudation of amino acids, organic acids, and sugars, which are the main source of food for growth and evolution of germinating mycorrhizal fungi spores (Ratnayake et al., 1978). Therefore, the impact of mycorrhizal fungi at high P level is limited.

Our finding is also in the same line with Abou El Seoud (2005) who found that carrot shoot yield treated with mycorrhizal strain 49 had highly response ascompared to shoot yield of other plants inoculated with AM strain 301 and the other carrot plants without mycorrhizae. All squash plants treated with different mycorrhizal strains obtained high root dry weight and root length at low P level (P0) as compared with untreated plants. The obtained results are in agreement with Abou El Seoud (2019). Similarly, Abou El Seoud (2008) who reported that, at low level of P, the tagetes root growth treated with mycorrhizal strain 49 increased significantly as compared to tagetes plants without mycorrhizae. Alves *et al.,* (2001) reported that developingplant root length under P stress could be the main possible mechanisms of P efficiency in maize. Phosphorus efficient of plants growing in low level of P in soil tends to have larger root system (Gaume et al., 2001). This mechanism is one of an adaptation of plants to enhance their uptake efficiency when P is a limiting growth factor (Föhse et al., 1988). The root length and root dry weight of plants with and without mycorrhizal strains inoculation increased with increasing P level. In contrast, Abou El Seoud (2005) found that the application of P in the soil had neutral impact on the root length of treated and untreated onion plants with mycorrhizal fungi. By other words, the root length of onion with and without mycorrhizae inoculation was reduced with increasing P application but insignificantly.

Among the mycorrhizal strains studied, (M301) led to improve root dry weight and root length of squash plants at low P level as compared to the other mycorrhizal strains and the control. There was no significant difference in all squash root growth (root dry weight and root length) between the two mycorrhizal strains (M49 and M139) at all P levels. This is in the same line with Abou El Seoud (2005) who obtained that no significant difference in carrot root length between the two mycorrhizal fungi strains (M49 and M301).

The present study indicated that plant P-uptake was improved by A-mycorrhizal fungi inoculation especially at low level of P in soil. These results agree with Abou El Seoud et al., (2018) who demonstrated that at low P level, there was a highly significant difference between plants with and without mycorrhizal inoculation in all wheat genotypes. Mycorrhizae may enhance nutrient uptake by reducing the distance between nutrients must diffuse and root system of plant (Liu et al., 2014). However, another explanation attributed the enhanced P-content to the increased total root system or efficiency in AM plants which would certainly contribute to increased total nutrients uptake. In this study, no significant difference was found in squash plant P uptake between the two mycorrhizal strains M49 and 139 at all P levels. In the same line, Abou El Seoud (2005) found that carrot P uptake which treated with strain 49 was improved by 28% than the other plant treated with strain 301 but there was no significant difference between the two strains.

Our finding of no significant difference in available P in soil between all mycorrhizal strains and uninoculated plants at high P level (P2) was previously reported by Smith and Smith (2011) who reported that increasing available P in soil lead to reduce mycorrhizal root colonization. In contrast, at low level of P supply, there was a highly significant difference between squash plants treated with all mycorrhizal strains especially mycorrhizal strain (M301) as compared with other squash plants without mycorrhizal inoculation. This result was in the same line with Nguyen et al (2019) who demonstrated that mycorrhizal fungi gave plants tolerance to P deficiency and this may be due to the motivation of the phosphate transporter (PT) genes (MtPT4) in the roots.

## Conclusion

Fromthe results and under the same conditions of this experiment, we recommended that all mycorrhizal strains were effective in improving plant growth. The strain M301enhanced squash plants growth and P uptake. Also, inoculation with this strain could improve P availability in soil. The two mycorrhizal strains (M49 and M139) can be used for improving the growth of squash plants under low P level and in calcareous soil conditions. This study is one of the few studies that indicate the specialization of mycorrhizal fungi strains on the host plant and this study must be followed by many other studies in different climatic conditions, different soil properties and on different genus and strains of AM fungi on different plants family to confirm or deny this hypothesis.

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## الميكوريزا يمكنها مساعدة نمو نبات القرع بالأرض الجيرية الفقيرة بالفوسفور

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يهدف هذاالعمل إلى اختيار سلالة اللقاح من فطر الميكوريزا لتعزيز نمو نباتات القرع في التربة الجيرية التي تعاني من نقصP.تم اختبار ثلاث معدلات من سماد فوسفات ثلاثى الكالسيوم بالتوازي، تم تلقيح الأصص ب 4 سلالات من فطر الميكوريزا 015. تم تكرار المعاملات 5 مرات فى تصميم قطاعات عشوائة كاملة وتم حصاد النباتات بعد 75 يومًا.الخلاصة، من النتائج وتحت نفس ظروف التجربة يمكن أن نوصى بأن جميع سلالات الميكوريزا كانت فعالة في تحسين نمو النبات. التلقيح بالسلالة M301 حسنت نمو نبات القرع وامتصاص الفوسفور ، أيضآ التلقيح بهذه السلالة أدى إلى تحسين الفوسفور المتاح بالتربة. يمكن استخدام سلالتي الفطر (M139 و M49) لتحسين نمونباتات القرع تحت مستوىP المنخفض وفي ظروف الأراضى الجيرية. هذه الدراسة هي واحدة من الدراسات القليلة التي تشير إلى تخصص سلالات فطر الميكوريزا على النبات المضيف ويجب أن تتبع هذه الدراسة العديد من الدراسات الأخرى في الظروف المناخية المختلفة، وخصائص التربة المختلفة وعلى أجناس وسلالات مختلفة من فطر الميكوريزا وعلى النباتات المختلفة لتأكيد هذه الفرضية أو رفضها.