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Response of Onion and Leek Plants during the Early Growth Stage to Mycorrhizal Inoculation on a Phosphorus Deficient Calcareous Soil

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Abstract: A pot experiment was carried out in the greenhouse at the faculty of Agriculture (Saba Basha), Alexandria University, Egypt, to evaluate the response of onion and leek plants during the early growth stage to mycorrhizal inoculation on a phosphorus (P) deficient calcareous soil. The soil used in this work was collected from Burg El-Arab City, Alexandria, Egypt from the surface layer to plough depth (down to 20 cm). Uniform seedlings of onion (*Allium cepa L.*) and leek (*Allium porrum L.*) each having two or three pairs of fully grown leaves, were selected and transplanted into the pots at the rate of two seedlings per pot. Half of the pots were inoculated with mycorrhizal fungi (M) (*Glomus intraradices*) while the other half was uninoculated (NM). All plants were harvested three times as 15; 25 and 35 days after transplanting (DAT). The experiment was structured following a randomized complete block design (RCBD) with five replications. The results showed that, under the same conditions of this experiment with P deficient calcareous soil, mycorrhizal fungi (AMF) varied in their ability to stimulate plant growth at different growth stages. Mycorrhizal inoculation negatively affected both onion and leek during the early two growth stages (0 – 15; 15 – 25 DAT) on the growth parameters (shoot dry weight; shoot height and root length). Contrarily, onion and leek growth were improved by mycorrhizal fungi in later growth stage (25 – 35 DAT) on the same growth parameters. The shoot dry matter, shoot height and root length of onion inoculated with mycorrhizal fungi at the third sampling date (35 DAT) increased by 32% ; 22% and 20% respectively compared to uninoculated onion plants. Also, the growth parameters of leek plants inoculated with AMF at the third sampling date (35 DAT) increased by 25%; 24.8% and 28% respectively compared to the uninoculated leek plants. On the other hand, the shoot P concentration and P uptake of onion and leek plants with and without Mycorrhizal fungi (AMF) increased with increasing plant growth stages. The increase in P concentration and P uptake of onion and leek plants inoculated with Mycorrhizal fungi was rapidly in the second growth stage (from 25 to 35 DAT) compared to the first growth stage (from 15 to 25 DAT). A highly significant correlation between root length and P uptake of plant (onion and leek with and without AMF) was observed. This study however indicated that beneficial responses due to mycorrhizal inoculation may need some time (35 DAT or more under the same conditions of this experiment) to develop its structure into the plant roots.

Key words: Calcareous soil, onion, leek, mycorrhizal fungi, early growth stage.

INTRODUCTION

A primary limitation of crop production in semi-arid regions is the deficiency of available nutrients especially phosphorus (P) (Nagarathna *et al.*, 2007). Phosphorus is a limiting element for plant growth particularly in low P soil of high Fe/Al or Ca contents, where P is strongly bound and largely unavailable for plant uptake (Marschner, 1995). Even if P was added to soil in soluble form, soon it becomes immobilized as organic P, calcium phosphates or other fixed forms (Turk *et al.*, 2006). In calcareous soils, phosphorus may be immobilized by any or all the following mechanisms: a) adsorption on active sites of $CaCO_3$ b) precipitation by Ca in the system resulting in reduced P availability C) reaction with the exchangeable Ca. The amounts of calcium carbonate affect distinctly the soil properties related to plant growth, whether they are physical, such as the soil water relations, and crusting, or chemical such as the availability of plant nutrients (FAO, 1984). In addition, at high pH (more than 8.0), the P in soil becomes unavailable.

The most realistic solution according to Marschner (1995) is the use of mycorrhizal fungi (AMF) that have the ability to acquire P and give high yield under limited P supply. An important impact of the AMF on plants is the alleviation of phosphate deficiency (Abou El seoud, 2005; Soil Health, 2010; Rouphael and Cardarelli, 2010 and Tawaraya *et al.*, 2012). Mycorrhizal fungi are the most widespread association between microorganisms and roots of higher plants. Several studies confirmed the contribution of mycorrhizae to P uptake efficiency. AMF inoculation might become very important for sustainable agronomical management, especially in cases when the efficiency of native inocula is poor (Pellegrino *et al.*, 2011). The hyphae of the fungus have the radius approximately 1.5 µm (Tinker *et al.*, 1992) and large surface area, leading to an increase of the P absorbing

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surface area, the production of organic acids and phosphatase which catalyze the release of P from organic complexes (Koide and Kabir, 2000 and Wang *et al*., 2004). Enhancement of P uptake by mycorrhizal hyphae can also be indirectly attributed to the faster uptake rate of P by the hyphae and the disturbance of the solution solid P equilibrium, which will increase the sorption of absorbed phosphate into soil solution (Nye and Tinker, 1977).

Plants with coarsely branch roots and with few or no root hairs are expected to be more dependent on mycorrhizal fungi than are plants with finely branched root systems (Smith and Read, 1997). Therefore, the selected plants which used in present study are dependent on mycorrhizal fungi to acquire P from the limited soil P, since onion (FÖhse *et al.,* 1991) and leek produce extremely short and stubby root hairs. That is help to study the contribution of mycorrhizal hyphae in acquisition P from the soil without any overlapping for root hairs in the total P uptake by plant. Onion and leek are most ancient and important vegetables known to man. Onion and leek are grown in just about every country in the world.

On the other hand, the beneficial responses due to mycorrhizal inoculation may need some time to develop its structure inside the plant root (Pharudi, 2010). Yang *et al.* (2010) reported that all inoculated seedlings were infected by arbuscular mycorrhizal fungi and formed arbuscular mycorrhiza after 30 days of co-cultivation. Spore germination was observed 3-5 days after surface sterilization as well as co-inoculation with transformed roots. Fungal growth was also recorded 2-10 days after inoculation. Fungal germinating hyphae branched and produced radical shape network 2.5mm in diameter. The first contact between fungus mycelium and roots occurred 1-3 days after germination. 7 days after fungus- host contact, several secondary spores or vesicle like structures observed which were similar to true spores except of their size (20- 30µm diameter). The first true spore formed 25 days after contact and then number of spores increased exponentially (Eskandari1 and Danesh, 2010).

The objective of this study was to evaluate the response of onion and leek plants during the early growth stage to mycorrhizal inoculation on a phosphorus deficient calcareous soil.

MATERIALS AND METHODS

A pot experiment was carried out in the greenhouse at the Faculty of Agriculture (Saba Basha) Alexandria University, Egypt.

Soil Preparation:

The soil used in this work was collected from Burg El-Arab City, Alexandria, Egypt from the surface layer to plough depth (down to 20 cm). The soil was air-dried, sieved through a 2 mm sieve to homogenize and separate roots from soil. The chemical properties of the soil (before starting the experiment) were as follows: pH (1:1 *w/v* water) 8.12, EC (1:1) 2.30 dS/m, organic matter 0.41%, $CaCO_3$ 26.18% , available nitrogen 17.20 mg/kg soil , available P 6.5 mg/kg soil (Olsen) , total P 0.45g/kg soil. The soil properties were determined according to the methods describing by (Black, 1965). Basal applications of N, K and Mg fertilizers were corporate with each kg soil at a rate of 150 mg N as NH_4NO_3, 150 mg K as K_2SO_4 and 40 mg Mg as $MgSO_4$ per kg soil. The N fertilizer was applied at three equal does at the rate of 50 mg/20 ml water for each pot. The K and Mg fertilizers were applied before filling the pots with soil.

Inoculum Preparation:

The mycorrhizal fungi (*Glomus intraradices*) were used in this experiment. The mycorrhizal specie was obtained from Hanover University, Germany and activated in the Soil Microbiology Lab., Soil and Agriculture Chemistry Dep., Faculty of Agriculture, Saba Basha, Alexandria University, Egypt.

Pot Experiment:

Each plastic pot (Ø = 15 cm and 12 cm deep) was uniformly filled with 1000 g of the prepared soil and compacted to bulk density of about 1.37 g cm^{-3}. The pots filled with soil were placed in a greenhouse. One week before planting, all pots were watered to the volumetric moisture content 0.25 cm^3cm^{-3}, which correspond to the field capacity. Uniform seedlings of onion (*Allium cepa L.*) and leek (*Allium porrum L.*) each having two or three pairs of fully grown leaves, were selected and transplanted into the pots at the rate of two seedlings per pot. Half of the pots (15 pots for each crop) were inoculated with AM fungi (M) while the other half was uninoculated (NM). In mycorrhizal pots, the soil was mixed with 20 ml mycorrhizae one week before planting as described by Malibari *et al.* (1990). Also, 10 ml inoculums were added with the seedlings at transplanting time, (in total, the rate of 500 spores per pot). All plants were harvested three times as 15, 25 and 35 days after transplanting. At each harvest time, shoots were separated from roots. Plant height was measured from the ground level to the tip of the tallest leaf (Fageria *et al*., 2006) using a meter ruler. The shoots were washed with tap water, distillated water. The shoots were then dried at 70ºC/48 hours (Steyn, 1959) to constant weight in a forced-draft oven for 48 hours and then

weighted (recorded) and milled for analysis. Samples of plant material were wet digested with H_2SO_4–H_2O_2 (Lowther, 1980). Phosphorus content was determined by vanadomolybdophosphoric method (Jackson, 1967).

Quantifying Roots Length:

Plant roots were removed from each pot and separated from soil by washing them several times under a jet of tap water on a 0.5 mm sieve to remove all the soil. Excess moisture was blotted from the cleaned roots by wrapping up the roots in layers of paper towel for 3 min (Schenk and Barber, 1979). For each pot three samples of 0.3 g fresh weight were used for determination of root length by the line intersect method according to Tennant (1975).

$$RL = \frac{11}{14} \times N \times G$$

Where:

RL = root length, N = sum of horizontal and vertical crossing, G = length of the grid unit (2 cm or 1 cm).The sample root length (0.3g) was converted to total root length per pot based on total fresh mass of the root in the pot.

Statistical Analysis:

The experiment was structured following a randomized complete block design (RCBD) with five replications. Data were analyzed by using analysis of variance in SAS (SAS institute INC. Cary, USA 1996). Tukey test was used to compare treatments means. A significance level of $\alpha = 0.05$ was used in all analysis.

RESULTS AND DISCUSSIONS

Plant growth:

Increased growth of plants inoculated with mycorrhizal fungi (AMF) is usually attributed to mycorrhizal colonization through increased capacity of plant roots to take up water and nutrients. In the present study, mycorrhizal inoculation of onion and leek plants did not have any significant effect at the first two sampling dates (after 15 and 25 days after transplanting (DAT)) on the growth parameters (shoot dry weight (Fig. 1); shoot height (Fig. 2) and root length (Fig. 3).

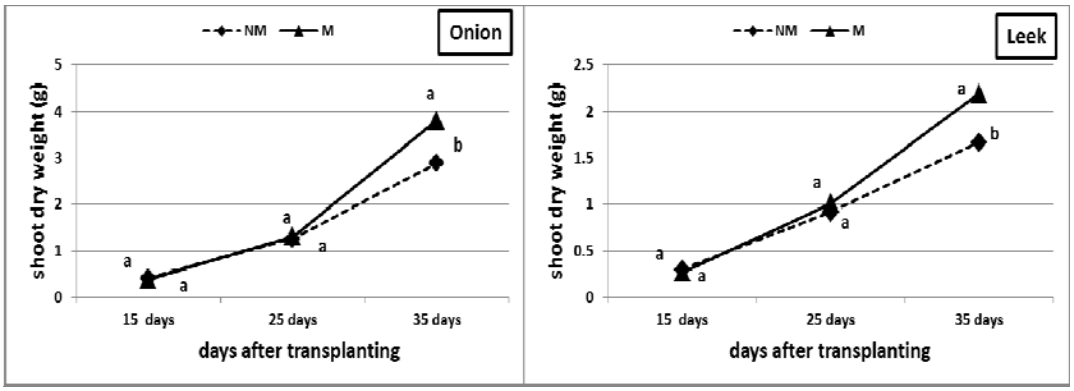

Fig. 1: Shoot dry weight (g) of onion and leek plants during the growth stages as affected by mycorrhizal fungi inoculation; different letters indicate significant differences, $p \leq 0.05$.

The absence of any positive response in mycorrhizal inoculation plants could be due to the fact that at the early growth stage (15 and 25 DAT in this study), the mycorrhiza was still forming an association with plant roots and did not yet improve the nutrient uptake of inoculated plants especially in calcareous soil which has low nutrient content. By other words, the absence of any response in mycorrhizal inoculated onion and leek plants might be due to that, AMF are known to be obligate fungal symbionts, they have to form an association with plant roots to survive and it may take some time for mycorrhiza to form this association (Turk, 2006). Therefore, onion and leek plants might not show any response to mycorrhiza during the early growth stage. In the same line, Pharudi (2010) reported that the wheat plants inoculated with AMF did not have any significant effect at the early growth stage on shoot fresh and dry weight, leaf area and root fresh weight compared to uninoculated plants. At the third sampling date (after 35 DAT), the onion and leek plants which inoculated with mycorrhizal fungi had a highly significant effect on the growth parameters, compared to uninoculated onion and

leek plants. Similarly, several researchers found that inoculation with AMF lead to enhance onion growth plants (Goussous and Mohammad, 2009; Wang *et al.*, 2011 and Tawaraya *et al.*, 2012) and leek growth plants (Sorensen *et al.*, 2008) than those of non-inoculated plants grown in P deficient soil. At the third sample date (35 DAT), increase in shoot growth of onion and leek plants inoculated with AMF could be due to an increase in the uptake of nutrients, especially P (Tinker, 1984). The mycorrhizal fungi extend a network of hyphae several centimeters out into the surrounding soil, thereby expending the effective volume of soil that plant can exploit (Frank, 2002). The hyphal mycelium increases the total absorption surface of infected plants and this improves its access of immobile elements such as P, Cu and Zn (Douds *et al.*, 2005 and Grant *et al.*, 2005). Also, at the third sample date (35 DAT); the root length of plant inoculated with mycorrhizal fungi was significantly longer than that of the other plants without mycorrhizal fungi. Similarly, Giri *et al.* (2005) found that root dry weight of root length of *Cassia siamea* was higher in mycorrhizal plants than non-mycorrhizal plants. Also, Neumann and George (2005) reported that, the root length of tomato was increased with inoculation with mycorrhizal fungi at low P level. Mycorrhizal fungi may increase the surface area of plant roots by increasing branches of first order lateral roots (Aguin *et al.*, 2004), which due to increase acquisition of P by increasing the extension of depletion zone around the root.

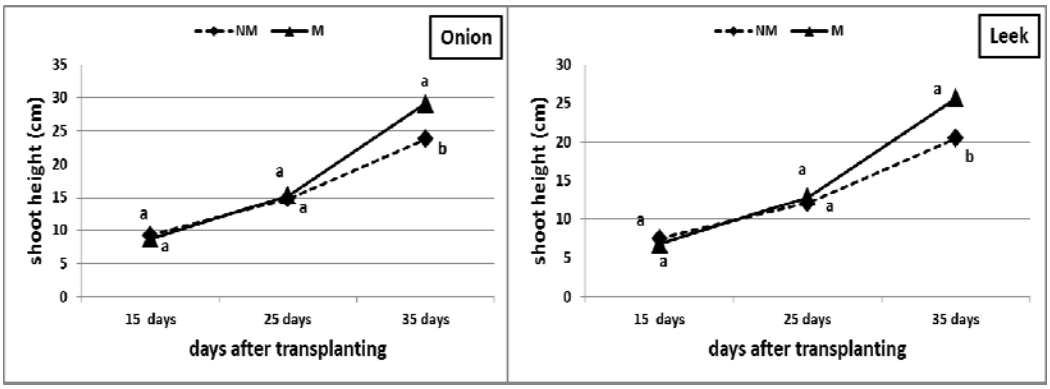

Fig. 2: Shoot height (cm) of onion and leek plants during the growth stages as affected by mycorrhizal fungi inoculation; different letters indicate significant differences, $p \leq 0.05$.

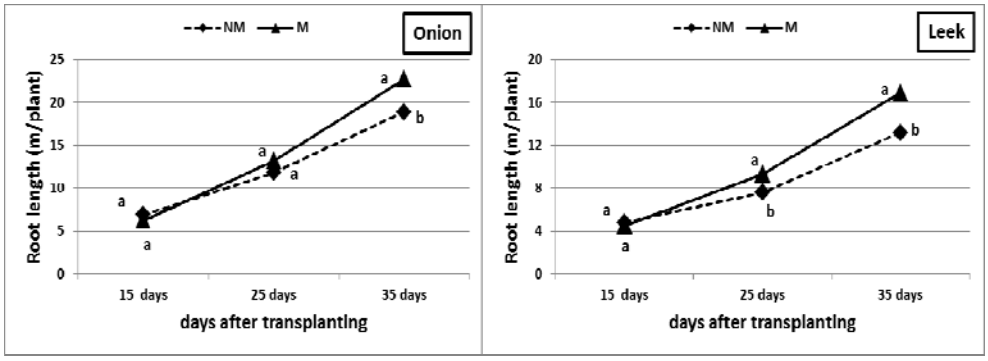

Fig. 3: Root length (m/plant) of onion and leek plants during the growth stages as affected by mycorrhizal fungi inoculation; different letters indicate significant differences, $p \leq 0.05$.

The shoot dry matter, shoot height and root length (Figs. 1, 2 and 3) of onion inoculated with mycorrhizal fungi at the third sampling date (35 DAT) increased by 32%; 22% and 20% respectively compared to uninoculated onion plants. Also, the growth parameters of leek plants inoculated with AMF at the third sampling date (35 DAT) increased by 25%; 24.8% and 28% respectively compared to the uninoculated leek plants. This response of onion and leek plants indicates that at 35 days after transplanting, the mycorrhiza has started to form an association with plant roots and was beginning to increase nutrient uptake by plants (Pharudi, 2010). Similarly, Bougher *et al.* (1990) stated that at low levels of soil P, height of karri seedlings inoculated with mycorrhizal fungi was similar to that of non-inoculated seedlings till about 35 days after planting, but started to accelerate after this initial period of growth. This observation supports earlier results (Khan, 1975 and Owusu-Bennoah and Mosse, 1979) who suggested that the positive effect of mycorrhizal inoculation becomes evident only after about one month. The same trend was obtained with Pharudi (2010) who found that, at 28

days after planting, maize plants from mycorrhizal inoculated seed had a significantly higher root fresh and dry weight, plant height, shoot fresh and dry weight and higher leaf area. In general, the results showed that mycorrhiza (AMF) varied in their ability to stimulate plant growth at different growth stages. On the other hand, several researchers reported that inoculation with AMF lead to enhance onion and leek plant growth (Abou El Seoud, 2005; Goussous and Mohammad, 2009 and Priyadharsini *et al.*, 2012). In contrast, non AMF onions were stunted due to P deficiency in the soil (Cantrell and Linderman, 2001). On the other words, mycorrhizal fungi enhanced shoot yield. This effect was pronounced at low P level. This is in line with the suggestion of Ning and Cumming, 2001; Cavagnaro *et al.*, 2003; Ortas, 2003 and Giri *et al.*, 2005. Similar result was observed in tagetes plant inoculated with mycorrhizal fungi attained more than 80% of its maximum yield at low available of P level (Abou El Seoud, 2008).

Phosphorus Content:

The shoot P concentration of onion and leek plants with and without Mycorrhizal fungi (AMF) increased with increasing plant growth stages (Fig. 4). The increase in P concentration of onion and leek plants inoculated with Mycorrhizal fungi was rapidly in the second growth stage (from 25 to 35 DAT) compared to the first growth stage (from 15 to 25 DAT). On the other hand, there was no significant difference between onion and leek plants with and without Mycorrhizal fungi on the first two sample dates (15 and 25 DAT). Suddenly, at the third sample date (35 DAT) the P concentration of onion and leek plants inoculated with AMF increased significantly compared to uninoculated plants. The shoot P concentration of onion and leek plants inoculated with AMF increased by 56% and 62.5% respectively compared to uninoculated onion and leek plants (Fig. 4), this led to increased shoot height and shoot dry weight (Sailo and Bagyaraj, 2005; Fan *et al.*, 2008 and Shamshiri *et al.*, 2012).

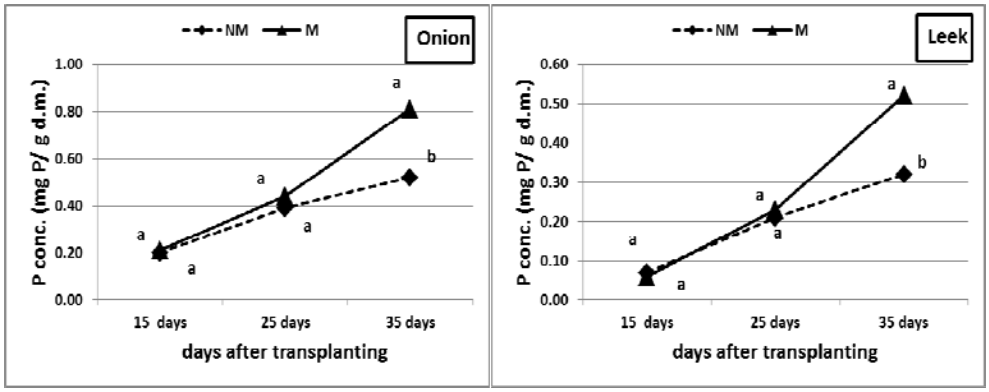

Fig. 4: Phosphorus concentration (mg P/ g d.m.) of onion and leek plants during the growth stages as affected by mycorrhizal fungi inoculation; different letters indicate significant differences, $p \leq 0.05$.

In the same line, Stephanie *et al.* (2011) and Ehteshami (2011) reported that arbuscular mycorrhizas (AM) play an important role in plant P content. Also, Tawaraya *et al.* (2012) reported that shoot P content of onion inoculated plants was higher than that of non-inoculated plants grown in P deficient soil. These results are in the same line with Abou El Seoud (2005); Abou El Seoud (2008) and Ehteshami (2011). On the other hand, the uptake of onion and leek plants was taken the same trend of P concentration. The P uptake of onion and leek plants with and without mycorrhizal fungi increased with increasing plants growth stage (Fig. 5). There was no significant difference in P uptake of onion and leek plants with and without AMF at the first two sample dates (15 – 25 DAT). In contrast, at the third sample date (35 DAT), there was huge significant difference in P uptake of onion and leek plants inoculated with AMF compared to the other plants without mycorrhizal inoculation (Fig. 5). At the third sample date (35 DAT), the P uptake of onion and leek plants inoculated with AMF were higher than uninoculated plants by about 105% and 104% respectively. Mycorrhiza may increase nutrient uptake by reducing the distance that nutrients must diffuse to plant roots (Abbott and Robson, 1982 and Abou El Seoud, 2005). Mycorrhizal roots due to their external hyphae that are capable of absorbing and translocating nutrients can explore more soil volume than the non-mycorrhizal roots and increase the supply of the slowly diffusing ions such as phosphate to the plant (Khaliq and Sanders, 2000). Also, the distribution of hyphae in soil zones where the roots are absent (the micro-sized cross-section of the hyphae facilitates the penetration of much smaller soil pore where roots cannot penetrate), as well as the bigger contact of the hyphae with the soil contributes largely to the increased nutrient and water uptake (Joubert and Archer, 2000).

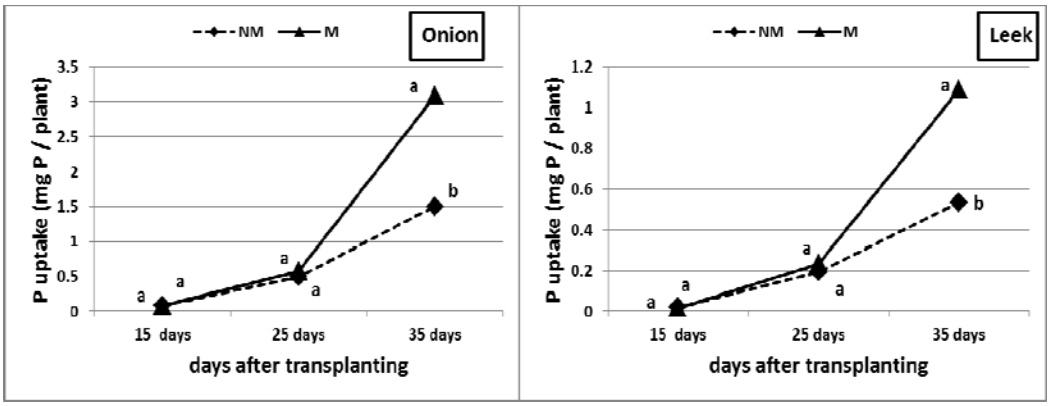

Fig. 5: Phosphorus uptake (mg P/ plant) of onion and leek plants during the growth stages as affected by mycorrhizal fungi inoculation; different letters indicate significant differences, $p \leq 0.05$.

On the same line, Al-Karaki *et al.* (2004) and Meyer (2007) reported that, AM plants enhanced uptake of immobile nutrients such as P, Zn and Cu particularly in soils with low P availability. Also, Tawaraya *et al.* (2012) stated that inoculation of onion plants with AMF increased shoot P uptake under P deficient soil conditions. The relationship between root length of onion and leek plants with and without AMF and P uptake was analyzed to determine the effect of root growth on the P uptake. A highly significant correlation of onion with and without AMF ($R^2 = 0.92$ and $R^2 = 0.98$, $p < 0.05$ respectively) between onion root length and P uptake was obtained (Fig. 6).

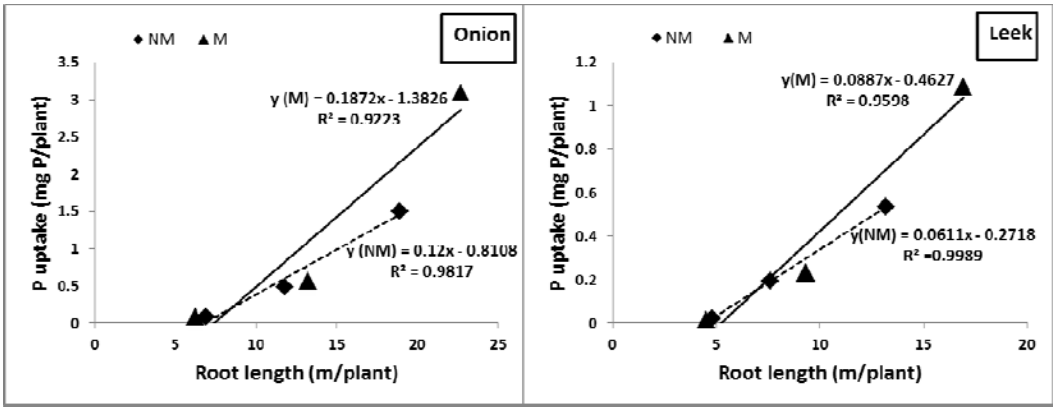

Fig. 6: Relationship between root length of onion and leek plants with and without mycorrhizal fungi and P uptake at different growth stages.

Also, a highly significant correlation of leek with and without AMF ($R^2 = 0.96$ and $R^2 = 0.998$, $p < 0.05$ respectively) between leek root length and P uptake was observed (Fig. 6). These results indicated that root growth contribute to the acquisition of P from the soil. The increase in P uptake is largely due to increased absorption of P from soil solution by plant's root. Similarly, Gao *et al.* (2005) and Abou El Seoud and Abdel-Megeed (2012) noticed the importance of root growth for nutrient acquisition.

Fig. (6) show that the correlation between root length and P uptake on onion and also leek was higher in non-inoculated plants than inoculated plants. This result could be due to the plants without mycorrhiza completely depends on their root growth to take up P and other nutrients and water from the soil. But in plants with AMF depends on other mechanisms beside the root growth, the extrametrical mycorrhizal hyphae which absorbing and translocating nutrients to associated plants (Abou El Seoud, 2005). Also, AMF may have biochemical capabilities for increasing the supply of available P and other immobile nutrients. These capabilities my involve increases in root phosphatase activity, excretion of chelating agents, and rhizosphere acidification (Habte and Fox, 1993).

In conclusion, under the same conditions of this study, the beneficial responses due to mycorrhizal inoculation may need some time to develop their structure into the root. During the early stages of growth (0 - 15 and15 - 25 DAT), both onion and leek were negatively affected by mycorrhizal fungi. Contrarily, onion and leek growth were improved by mycorrhiza in later growth stage (25 - 35 DAT) which indicate that mycorrhiza

have formed an association with plant roots and therefore enhanced P uptake by plant roots. In general, mycorrhizal inoculant is environment – friendly nutrient supplement for onion and leek and should be included in onion and leek production system mostly with low available phosphorus, but the positive effect of AMF inoculated with onion and leek plants becomes evident only after 35 days or more.

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