# Could the artificial inoculation of AM fungi improve the benefits of using pea (*Pisum sativum* L.) plants for soil amendment purposes in greenhouses?

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***Abstract***

**The study aimed to investigate the effects of commercially available AMF inoculate (*Glomus* sp. mixture) on the growth and the nutrient acquisition of garden pea (*Pisum sativum* L.) plants. Inoculated (AMF+) and non-inoculated (AMF-) pea plants were subjected to two levels of NaCl salinity (0 and 50 mM NaCl). Several times during the growing cycle, randomly selected plants were analyzed for dry matter of roots and the aboveground biomass. Plant tissue samples were analyzed for N, P and K concentration and the total uptake, specific absorption rate (SAR) and specific utilization rate (SUR) of these elements were calculated. Raised salinity drastically decreased the dry matter of roots and aboveground biomass and significantly deteriorated the specific utilization rate (SUR) of main nutrient elements in pea plants. The presence of AM fungi significantly reduced the dry matter of roots, but on the contrary significantly increased the dry matter of shoots and the overall plant dry matter. Furthermore, AM fungi inoculation significantly increased the specific absorption rate (SAR) of N, P and K under both; control and saline conditions and enhanced the specific absorption rate (SUR). As a conclusion, the inoculation of garden pea with AM fungi significantly increased the volume and improved biomass production in pea grown as greenhouses soil amendment crop (green manure).**

**Keywords:** mychorrhizae, nutrient uptake, specific absorption rate, specific utilization rate

## INTRODUCTION

Since organic farming is a system that excludes the use of synthetic fertilizers, organic greenhouse farmers heavily rely on crop rotations, crop residues, animal manures, legumes, green manures, organic wastes, and mineral-bearing rocks to feed the soil and supply plant nutrients (Greer and Diver, 2000). Though pea (*Pisum sativum* L.) is grown essentially for its protein-rich seeds (Tayeh et al., 2015a), it is also an essential component of sustainable cropping systems, due to its ability to develop symbiotic nitrogen fixation as well as its role as a break crop for pest and pathogen reduction (Tayeh et al., 2015b). Growing pea in organic greenhouses would improve greenhouse soil quality by enhancing the N-supplying power of soils, increasing the soil reserves of organic matter, stimulating soil biological activity, improving soil structure, increasing soil aeration, improving soil water-holding capacity and making the soil easier to till (Biederbeck et al., 2005). Though the main effect of legumes as green manure is to add nitrogen-rich, readily decomposable plant material, they also greatly stimulate the activity of soil microbes and, as a result, speeds up the cycling of nutrients (Biederbeck et al., 2005).

Salinity is a common and most severe environmental stressor in agriculture (Shelden and Roessner, 2013; Flowers, 2004), which is dramatically exacerbated by irrigation (Carillo et al., 2011). Soil salinization due to poor water quality is a serious threat, especially under protected cultivation, where natural leaching of excess salts by rain water is absent. Additionally, erroneous fertilization schemes contribute to salt accumulation in plant rooting zone and rapid degradation of soil chemical and physical properties (Balliu et al., 2015).

Under these circumstances, the use of commercial inoculants containing arbuscular

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mycorrhizal fungi (AMF) is quickly expanding, rewarded as an environmentally friendly technology which contributes to the alleviation ofthe negative effects of soil/irrigation water salinity. Considering that most legumes are rather sensitive to salinity, and only a few legumes can grow in salt-affected soils (Egamberdieva et al., 2013), the objective of this study was to assess the impact of AMF inoculants containing a mixture of AM fungi (*Glomus intraradices*, *Glomus etunicatum*, *Glomus mosseae*, *Glomus geosporum*, and *Glomus clarum*) on plant growth parameters and nutrient absorption capacity of garden pea (*Pisum sativum* L.) grown as green manure crop in organic greenhouses, under normal and saline conditions.

**MATERIALS AND METHODS**

The experiment was conducted in a non heated greenhouse at Agricultural University of Tirana, Tirana, Albania. For that purpose, graded seeds of a commercial pea cultivar (Progress 9) were sown in large plastic pots (0.6×0.2×0.2 m) filled either with i) vermiculite (Agra-Vermiculite, Pull Rhenen B.V., The Netherlands) + peat moss (Potground H) (vol:vol; 2:1) and 10% (vol/vol) crushed, expanded clay particles coatedwith AM-fungal spores (~200 spores $g^{-1}$; mixture of *Glomus intraradices*, *Glomus etunicatum*, *Glomus mosseae*, *Glomus geosporum*, and *Glomus clarum*; AMF+), or ii) vermiculite + peat moss (2:1) with crushed, spore-free expanded clay particles (10% vol/vol); AMF-. The clay particles with/without AMF spores were supplied by BioSym B.V. (Hengelo, The Netherlands) and homogenously mixed with the substrate before sowing. To each pot, 40 graded seeds, sown 2 cm apart from each other at 2 cm depth, in two parallel lines. The seeds were sown in December 10, 2015 and the experiment lasted till May 30, 2015.

Two different levels of salt-stress (0 and 50 mM NaCl) were established by the addition of different amounts of sodium chloride (NaCl) to the irrigation water. The non-inoculated (AMF-) and inoculated (AMF+) plants were equally distributed to both salinity treatments, according to a full factorial design. Consequently, four treatments, i.e., control (AMF-, 0 mM NaCl), salt stress without mycorrhizal inoculation (AMF-, 50 mM NaCl), mycorrhizal only (AMF+, 0 mM NaCl), and salt stress with mycorrhizal inoculation (AMF+, 50 mM NaCl) were established. Each treatment was represented by 7 pots placed in row alongside each other; each of them represented a replication.

Plants were watered during the whole experimental period with equal amounts of either tap water (0 mM NaCl), or saline water (50 mM NaCl).The irrigation was conducted by a gravity driven drip irrigation system (2 drippers $pot^{-1}$, with 0.2 L $h^{-1}$ discharge rate). For that purpose, individual 200-L deposits were placed over a 2 m high platform. The time, frequency and length of irrigation cycles were automatically controlled by an electronic irrigation controller (Itec 8, Netafim Ltd., Israel).

At DAS (day after sowing) 18, 42 and 58, 10 plants of each treatment were randomly selected and harvested. Roots were gently washed free of adhering substrate particles, and plants were dissected and separated into roots and stems. The plant organs were subsequently dried (65°C, 48 h) and weighted separately to an accuracy of 0.001 g (TP 303; Denver Instruments GmbH, Göttingen, Germany). The whole plant material (roots + shoots) was mixed together, grounded and analyzed for nutrient content (N, P, K), respectively at DAS 18, 42 and 58. The total uptake of each nutrient accumulated in the plant was calculated as the product of leaf dry matter and nutrient concentration (Martinez et al., 2005; Huang et al., 2013).

$$\text{X accumulation (mg)} = \text{X concentration (mg } g^{-1}) \times \text{plant dry weight (g)} \quad (1)$$

Following that, the specific absorption rate (SAR; g $mg^{-1}$ $d^{-1}$) as the indicator of root absorption efficiency (Martinez et al., 2005), was calculated as:

$$(X_{t2} - X_{t1})/(t2 - t1) \times (\ln w_{t2} - \ln w_{t2})/(w_{t2} - w_{t1}) \quad (2)$$

and specific utilization rate (SUR; g $mg^{-1}$ $d^{-1}$), as the index of the efficiency of a certain nutrient (N, P, K) in producing biomass is the rate of plant biomass production per unit of the

nutrient in the leaves (Martinez et al., 2005) was also calculated as:

$$(Wt2 - Wt1)/(t2 - t1) \times (lnXt2 - lnXt1)/(Xt2 - Xt1) \quad (3)$$

where: X1 and X2 is plant nutrient content (mg) at the start and at the end of analyzed period, W is plant dry matter ($DM_{Plant}$) and w is the root dry weight ($DM_{Root}$), t2 = end of analyzed period (DAS 18), and t1 = start of analyzed period (DAS 52).

Differences in DM, nutrient concentrations, SAR and SUR of specific elements were tested between by two way ANOVA, using the PC program StatPlus 2009 v5 (AnalystSoft Inc., Walnut, CA, USA). Each significant ANOVA result ($p<0.05$) was followed by an LSD tests at $p<0.05$, as post-hoc test to compare pair wise means within and among treatments. Values given throughout the text are means ± SD.

## RESULTS AND DISCUSSION

There are numerous publications (Cuartero et al., 2006; Huang et al., 2009; Edelstein et al., 2011; Porcel et al., 2012; Colla et al., 2012; Balliu et al., 2015) confirming that plants' growth decreases with increasing salinity. Similarly, in this experiment the presence of NaCl in irrigation water has drastically reduced the growth of pea plants, both, the root system and the above ground plant biomass. Though soon after the exposure of pea plants to saline water (18 DAS) no significant differences were found between control and saline plants regarding the dry matter of root system ($DM_{Root}$), the differences became statistically significant 42 days after sowing and further gradually enlarged (Table 1). Consequently, 58 days after sowing, the average dry mater ($DM_{Root}$) of the root system in saline plants was only 0.102 g versus 0.172 g in the control (non saline) plants. Similarly, a significant and gradually enlarged difference was found between control and saline plants regarding the dry matter of aboveground biomass ($DM_{Shoot+Leaves}$) (Table 2). 58 days after sowing, the average dry matter of aboveground biomass in saline plants was only 1.554 versus 2.442 g plant$^{-1}$ in control plants.

Table1. Dry matter (g) of the root system ($DM_{Root}$) of pea plants with/without arbuscular mycorrhizal fungi (AMF+/AMF-) and under two levels of salinity (0 and 50 mM NaCl). Different letters indicate significant differences within following parameters (Fisher LSD test, $p<0.05$; mean±SE).

| Sources of variation | | Day after sowing (DAS) | | |
|---|---|---|---|---|
| | | 18 | 42 | 58 |
| AM fungi | AMF- | 0.018±0.004b | 0.063±0.022a | 0.170±0.048a |
| | AMF+ | 0.021±0.005a | 0.051±0.019b | 0.104±0.035b |
| Salinity (S) | 0 mMNaCl | 0.019±0.005 | 0.074±0.016a | 0.171±0.048a |
| | 50 mMNaCl | 0.020±0.004 | 0.040±0.008b | 0.102±0.032b |
| Interaction AMF × S | AMF- × 0 | 0.016±0.003b | 0.084±0.008 | 0.209±0.038 |
| | AMF- × 50 | 0.020±0.004a | 0.043±0.008 | 0.131±0.011 |
| | AMF+ × 0 | 0.023±0.004a | 0.065±0.018 | 0.134±0.019 |
| | AMF+ × 50 | 0.019±0.005ab | 0.037±0.007 | 0.073±0.009 |
| Significance | | | | |
| AMF | | * | * | *** |
| S | | ns | *** | *** |
| AMF × S | | ** | ns | ns |

That increasing deleterious effects of salinity over the plant growth could be explained by the two-phase growth response to salinity (Munns, 2002). According to Munns (2002), the first phase of growth reduction is essentially a water stress or osmotic phase, and the growth reduction is presumably regulated by hormonal signals coming from the roots. Then there is a second phase of growth reduction, which is due to salts accumulation in

transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize salts in the vacuole. The consequences of $Na^+$ or $Cl^-$ build-up in the cell walls are often catastrophic and include dehydration and oxidative stress (Munns, 2002; Fatemi, 2014), finally causing leaf dieback. If the rate of leaf dieback will be faster than the rate of leaf expansion, the consequence will be the decrease of root dry matter and gradually deteriorated status of plant's water and nutrient supply.

Table 2. Dry matter (g) of the aboveground part ($DM_{Shoots+Leaves}$) of pea plants with/without arbuscular mycorrhizal fungi (AMF+/AMF-) and under two levels of salinity (0 and 50 mM NaCl). Different letters indicate significant differences within following parameters (Fisher LSD test, $p<0.05$; mean±SE).

| Sources of variation | | Day after sowing (DAS) | | |
|---|---|---|---|---|
| | | 18 | 42 | 58 |
| AM fungi | AMF- | 0.052±0.007 | 0.281±0.039b | 1.954±0.481b |
| | AMF+ | 0.050±0.008 | 0.352±0.093a | 2.142±0.755a |
| Salinity (S) | 0 mM NaCl | 0.056±0.007a | 0.366±0.083a | 2.442±0.510a |
| | 50 mM NaCl | 0.046±0.006b | 0.267±0.026b | 1.554±0.340b |
| Interaction AMF × S | AMF- × 0 | 0.058±0.005 | 0.297±0.042b | 2.140±0.533a |
| | AMF- × 50 | 0.047±0.004 | 0.265±0.032b | 1.767±0.377b |
| | AMF+ × 0 | 0.054±0.008 | 0.434±0.046a | 2.744±0.265a |
| | AMF+ × 50 | 0.046±0.007 | 0.269±0.023b | 1.341±0.057b |
| Significance | | | | |
| AMF | | ns | *** | * |
| S | | *** | *** | *** |
| AMF × S | | ns | *** | ** |

Arbuscularmycorrhizal fungi (AMF) have been frequently reported to improve crop plants' tolerance to stressful abiotic environments such as saline soils, though AMF themselves can be negatively affected by soil salinity (Jahromi et al., 2008). Many reports confirm the improvement of growth and performance of mycorrhizal plants under salt stress (Abdel Latef and Chaoxing, 2011; Porcel et al., 2012; Tüzel et al., 2012; Huang et al., 2013). Though we did not find any positive effects of AM fungi regarding the dry matter of root system, the presence of AM fungi has significantly increased the dry matter of aboveground plant part ($DM_{Shoot+Leaves}$). While no immediate affect was found till 18 days after sowing, the average dry matter of the aboveground part in AMF+ plants 58 days after sowing reached 2.142 versus 1.954 g $plant^{-1}$ in AMF- plants (Table 2). Anyway, the advantage of mychorrhized plants over AMF- regarding new biomass produced was significant only in non-saline conditions; no effect was found under severe salinity conditions (Table 2).

The symbiosis of plants with AM fungi often results in increased nutrient uptake(Waddington, 2003; Abdel Latef and Chaoxing, 2011; Evelin et al., 2012; Balliu et al., 2015). Many authors link the role of AM fungi in salinity stress alleviation with the increased uptake rate of P and K (Al-Karaki, 2006; Evelin et al., 2009; Abdel Latef and Chaoxing, 2011) by developing an extensive hyphal network and the higher hyphae affinity to a lower threshold concentration for absorption than in plant roots (Evelin et al., 2009, 2012). We analyzed the specific absorption rate (SAR) as an indicator of root absorption efficiency (the daily amount of nutrient absorbed per unit of dry matter of roots; mg $g^{-1}$ $d^{-1}$) and also found a highly significant improvement due to AM fungi presence regarding N, P and K absorption rate (Table 3). The respective calculated values in AMF+ plants were more than twice higher than in AMF- plants (Table 3). Interestingly, though the absorption capacity of roots due to AM fungi was significantly enhanced either in non-saline or severe salinity conditions, the differences were more distinguished under saline conditions (Table 3).

Table 3. Specific absorption rate (mg $g^{-1}$ $d^{-1}$) of N ($SAR_N$), P ($SAR_P$) and K ($SAR_K$) of pea plants with/without arbuscular mycorrhizal fungi (AMF+/AMF-) and under two levels of salinity (0 and 50 mM NaCl). Different letters indicate significant differences within following parameters (Fisher LSD test, $p<0.05$; mean±SE).

| Sources of variation | | $SAR_N$ | $SAR_P$ | $SAR_K$ |
|---|---|---|---|---|
| AM fungi | AMF- | 11.6±3.79b | 3.39±1.13b | 5.76±1.85b |
| | AMF+ | 31.5±12.6a | 7.56±1.98a | 12.1±3.00a |
| Salinity (S) | 0 mM NaCl | 15.5±7.53b | 4.59±2.30b | 7.67±3.66b |
| | 50 mM NaCl | 27.6±15.9a | 6.36±2.77a | 10.1±4.18a |
| Interaction AMF × S | AMF- × 0 | 9.21±3.75d | 2.64±1.07d | 4.61±1.87d |
| | AMF- × 50 | 14.0±1.88c | 4.15±0.55c | 6.91±0.94c |
| | AMF+ × 0 | 21.8±3.78b | 6.55±1.13b | 10.7±1.84b |
| | AMF+ × 50 | 41.2±10.6a | 8.57±2.21a | 13.4±3.47a |
| Significance | | | | |
| AMF | | *** | *** | *** |
| S | | *** | ** | * |
| AMF × S | | ** | ** | ** |

Table 4. Specific utilization rate (g $mg^{-1}$ $d^{-1}$) of N ($SUR_N$), P ($SUR_P$) and K ($SUR_K$) of pea plants with/without arbuscular mycorrhizal fungi (AMF+/AMF-) and under two levels of salinity (0 and 50 mM NaCl). Different letters indicate significant differences within following parameters (Fisher LSD test, $p<0.05$; mean±SE).

| Sources of variation | | $SUR_N$ | $SUR_P$ | $SUR_K$ |
|---|---|---|---|---|
| AM fungi | AMF- | 0.0026±0.00a | 0.0090±0.00 | 0.0052±0.00 |
| | AMF+ | 0.0023±0.00b | 0.0091±0.00 | 0.0055±0.00 |
| Salinity (S) | 0 mMNaCl | 0.0028±0.00a | 0.0096±0.00a | 0.0054±0.00 |
| | 50 mMNaCl | 0.0022±0.00b | 0.0085±0.00b | 0.0053±0.00 |
| Interaction AMF × S | AMF- × 0 | 0.0025±0.00b | 0.0090±0.00b | 0.0051±0.00 |
| | AMF- × 50 | 0.0027±0.00b | 0.0091±0.00b | 0.0053±0.00 |
| | AMF+ × 0 | 0.0030±0.00a | 0.0103±0.00a | 0.0058±0.00 |
| | AMF+ x 50 | 0.0016±0.00c | 0.0080±0.00b | 0.0053±0.00 |
| Significance | | | | |
| AMF | | ** | ns | ns |
| S | | *** | ** | ns |
| AMF × S | | *** | ** | ns |

The negative effects of raised salinity on plant growth were also expressed in respective smaller values of specific utilization rate (SUR; g $mg^{-1}$ $d^{-1}$) of N and P. Salinity has significantly reduced SUR values of N and P, means that compared to control plants, a significantly smaller biomass (dry matter) was created in saline stressed plants for each existing unit (mg) of N or P in plant leaves. Different from them, no effect was found regarding specific utilization rate of K (Table 4). We found that AM fungi have significantly raised the specific utilization rate of N and P in non-saline conditions, which fits well with conclusions of Porcel et al. (2012) and Rewald et al. (2015). They have reported that AM fungi enhance plant growth through; accumulation of osmoregulatory compounds, increasing photosynthetic rates, and decreasing root respiration and water use, which overall increase the efficiency of use of mineral nutrients in plant' leaves. Contrary to that, no positive effect of AM fungi presence was found regarding the specific utilization rate of main nutrient elements under salinity conditions. Since, significantly smaller SUR values were found regarding N and P in mychorrhized plants under salinity conditions (Table 4), we can assume that a considerable amount of plant carbohydrates addressed to the root

system of mychorrhized plants was used by AM fungi. Therefore, a significant reduction of root dry matter of mychorrhized plants was proved. However, in expense of that, AM fungi have increased the specific absorption rate of the root system regarding N, P and K.

## CONCLUSIONS

Saline irrigation water strongly diminishes the growth of pea plants and strongly reduces the absorption capacity of its root system. The inoculation of AM fungi in the growing substrate could alleviate salinity stress effects by increasing plant biomass and improving the absorption capacity of plants' root system. Therefore, the artificial inoculation of AM fungi could be considered as an effective and environmentally friendly alternative to significantly increase the volume and improve the quality of biomass in pea plants grown for greenhouses soil amendment (green manure) purposes.

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