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Comparative Effect of Two Arbuscular Mycorrhizae and N and P Fertilizers on Growth and Nutrient Uptake of Onions

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ABSTRACT

A pot experiment was conducted to investigate the effect of two types of mycorrhizal fungi (AM1, a mixture of indigenous mycorrhizal fungi & AM2, *Glomus intraradices* Schenck & Smith) and NP fertilizers on onions. Onion (*Allium cepa* L.) seedlings were grown in plastic pots containing these soils. Eight weeks later, they were evaluated for AM-colonization, growth characteristics and nutrient contents. AM-colonization was in the order of AM1>AM2>NP>control. NP plants showed the highest increase in dry biomass (71%) followed by AM1 (46%) and AM2 plants (14%). All treatments produced higher P content (about 100%) above control, but only NP and AM1 plants displayed a higher N content (56 & 60%, respectively). Shoot Zn was increased to 35 - 38% in both AM1 and AM2 plants, but not in NP plants. All treatments led to a higher Fe content with AM1 being highest. Only AM1 fungi produced an increase in Cd uptake. No effect was observed on Cu and Mn content in any treatment. Soil analysis at the end of experiment indicated no change in pH or electrical conductivity, but elevated P level in all test soils. This study indicates that inoculation with AM fungi, especially indigenous types is comparable to NP fertilizer application in enhancing onion growth and thus could provide a sustainable and environmentally safer option.

Key Words: *Glomus intraradices*; Greenhouse; Green onions; Jordan; Macronutrients; Micronutrients; Mycorrhiza

INTRODUCTION

Arbuscular mycorrhiza (AM) is a group of obligate fungi that lives in a symbiotic relationship with the roots of most agricultural crops (Smith & Read, 1997; Giovannetti *et al.,* 2006; Javaid, 2007; Javaid *et al.*, 2007; Javaid & Riaz, 2008). These fungi form essential components of sustainable soil-plant systems and improve crop growth and productivity (Van Der Heijden *et al.*, 1998; Schreiner *et al.*, 2003; Kapoor *et al.,* 2004; Cavagnaro *et al.,* 2006). They benefit their host plants by improving nutrient uptake like phosphorus (P), nitrogen (N) and micronutrients (Barea *et al.*, 1991; Clark & Zeto, 2000; Ward *et al.*, 2001; Javaid, 2009). AM fungi also provide their host plants with protection against environmental abiotic stresses (Augé, 2001; Javaid, 2007; Azcón *et al.,* 2009) as well as, biotic stress (Khaosaad *et al.,* 2007). The benefits of artificially inoculating a wide variety of agronomic plant species with AM fungi have been documented in numerous studies. Nowadays, their application as a biofertilizer in crop production is recommended with the aim of increasing productivity and reducing fertilizer use (Schwartz *et al.*, 2006). Benefits, however, are not guaranteed and the factors that determine the efficiency of this fungal association are still unclear. Some of these factors were suggested to be AM and plant species, competition with other soil micro-organisms as well as, the nutrient status of the soil (Sylvia *et al.*, 2005).

Onion (*Allium cepa* L.) is one of the leading vegetable crops worldwide. In the Middle East, onion is used as bulbs or harvested earlier and consumed as green leaves. It constitutes a major part of daily diet as it is included in almost all recipes. Due to its superficial root system that is rarely branched and lacks root hairs, onion is very inefficient in the uptake of water and nutrients. As a result, large amounts of chemical fertilizers are usually used in onion cultivation. The use of chemical fertilizers, however, has its negative side. In general, chemical fertilizers are expensive, produce short-term benefits and above all, their use may contribute to environmental pollution. Therefore, attempts have been directed towards minimizing dependence on chemical fertilizers. For onion production, one way to do so would be the use of AM fungi. Previous research indicated that onion is highly responsive to several AM fungi, which tend to associate with onion roots leading to improved plant growth and nutrient uptake as well as, to increased tolerance to soil salinity and water stress (Mahaveer & Alok, 2000; Bolandnazar *et al.*, 2007a & b). However, results of these studies were variable depending on AM species, soil fertility and experimental conditions.

The present study was undertaken to assess the effect of two types of AM fungi; AM1, an indigenous AM mixture isolated from native soil and AM2, a commercial preparation of *Glomus intraradices* Schenk and smith; on the growth and nutrient uptake of green onions and to

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compare the performance of these fungi with chemical fertilizer application.

MATERIALS AND METHODS

Soil analysis and treatment. Upper surface soil (top 30 cm) was collected from the campus of Jordan University of Science and Technology (JUST) localized in Irbid, Jordan. The soil was air dried and sieved through 5 mm screen. Soil samples were analyzed for pH, electrical conductivity (in 1:1 soil extract in distilled water) (Rhoades, 1982). The P content of the soil was estimated by extraction with $NaHO_3$ (Murphy & Riley, 1962) and organic matter content by rapid oxidation (Nelson & Sommers, 1982). Micronutrients contents of the soil were determined in the DTPA soil extraction by the atomic absorption spectrophotometer (Linday & Norvell, 1978). Finally, soil texture analysis was performed by the hydrometer method (Gee & Bauder, 1986). The major characteristics of this soil are presented in Table I. In general, it is characterized as being basic, alkaline and poor in organic matter, N and P. The soil K content was found to be relatively high and adequate for normal plant growth (Mohammad, 2004).

Before use in the experiment, the soil was fumigated with methyl bromide under air-tight plastic sheets and was left for one week for the fumigant to dissipate. Soil analysis for pH, electrical conductivity and P content was also done at the end of experiment after harvest.

Fungal inocula. Two types of fungal inocula were used in this experiment. The first, AM1 was a mixture of indigenous AM fungi that was isolated from a field in north Jordan and the second, AM2 was a subculture of the commercial AM *Glomus intraradices*. The latter inoculum was supplied by Dr. M. Al-Momani (University of Jordan, Amman).

The two isolates were multiplied in the greenhouse in pot cultures on chickpea as a suitable host using sterile sand and soil in 1:1 ratio. Ten gram inoculums consisting of infected root fragments and adhering rhizosphere soil were used as inocula. Before inoculation, mycorrhizal colonization was assessed on chickpea roots. Roots with 50% colonization and rhizosphere soil with sporulation of about 50 ± 5 spores g^{-1} were used as inoculum. Control and NP treatments received the same quantity of non-mycorrhizal chickpea root fragments and rhizosphere soil.

Plant treatment and cultivation. The experimental treatments in this experiment were: (i) AM1, soil inoculated with AM1, (ii) AM2, soil inoculated with AM2, (iii) NP, soil mixed with N and P fertilizers at the rate of 125 mg N kg^{-1} soil as ammonium sulfate and 30 mg P kg^{-1} as triple super phosphate and (iv) control, soil alone with no fungal or fertilizer addition. The last two treatments received non-mycorrhizal inocula described above. Potassium was not applied to NP treatment due to the high soil K content. Experimental units consisted of plastic pots (diameter 22 cm) containing 4 kg of fumigated soil. In each pot, about 3 kg were placed at the bottom. On top, one of the three inoculum types was added and then covered over with the remaining soil. The soil in these pots was watered with distilled water to field capacity and equilibrated. Three weeks old onion seedlings (cv. Texas Grano) raised in a sterile nursery were transplanted to these pots at the density of three plants per pot. The pots were placed in the glasshouse under natural illumination and 26.5 and 10.5°C mean maximum and minimum temperatures, respectively. They were arranged in a randomized complete block design with 5 replicates per treatment. Plants were daily watered with deionized water without fertilizer.

Plant harvesting and analysis. Plants were harvested 8 weeks after transplanting. They were uprooted and the shoots were separated, weighed and their height measured. These shoots were then oven dried (1 week, 70°C) and their dry weight was determined. Plant leaf tissue samples were analyzed for P content by the vanadate-molybdate-yellow method (Chapman & Prat, 1982) and for N by the Kjeldahl method (Bremner & Mulvaney, 1982). The trace elements (Zn, Cd, Fe, Cu, & Mn) contents of the digestate were measured by atomic absorption spectrophotometry (Linday & Norvell, 1978).

Estimation of root AM colonization. To examine the presence of mycorrhizal fungi, roots of each plant were washed free of soil and 1 g subsamples were randomly taken and fixed in a formaldehyde-acetic acid-ethanol solution (90:5:5; by volume). Using a modified method of Phillips and Hayman (1970), root samples were cleared in 2% KOH, stained in 0.1% trypan blue and cut into 1 cm segments. From each root sample, 10 randomly selected root segments were arranged parallel to each other on a microscope slide and the mycorrhizal root infection (hyphae, vesicles & abuscules) was determined microscopically at 100X (Bierman & Linderman, 1981). Five vision fields were examined in each 1 - cm root section. The root infection percentages (colonization) were calculated as a ratio of the infected to the total sections examined.

Statistical analysis. All data were subjected to analysis using SAS program. Probability of significant was used to indicate significant treatments effects. Means were separated according to the Fisher's Least Significant Difference (LSD) at 0.05 levels of probability.

RESULTS

AM root colonization and growth response. Root colonization by AM fungi occurred in all treatments including non-inoculated control plants (Table II). The latter plants indicated a colonization level of 18%. In the other three treatments, colonization was significantly higher than this value and came in the order of AM1 (54) > AM2 (42%) > NP (24%). Differences among these treatments were significant.

The growth of onion plants was enhanced significantly by all three treatments; NP, AM1 and AM2 (Table II). NP plants had the highest biomass as reflected in a 58 and 71%

Table I. Selected characteristics of the surface soil used in the experiment

Surface soil characteristics	Value
pH	8.0
EC (dS m^{-1})	0.62
N-NH_4 (g kg^{-1})	6.34
N-NO_3 (g kg^{-1})	14.7
P (g kg^{-1})	7.34
K (g kg^{-1})	564
Ca CO_3 (%)	12.6
Organic matter (%)	0.81
Sand (%)	5.5
Silt (%)	61.0
Clay (%)	33.5

Table II. Effect of mycorrhizal inoculation and NP fertilizer application on AM root colonization and growth parameters of onion plants

Treatment	AM colonization (%)	Shoot fresh weight (g)	Shoot dry weight (g)	Plant height (cm)	Number of leaves per plant
Control	18.18 d	16.44 d	1.78 d	26.00 c	4.89 a
NP fertilizers	24.15 c	25.97 a	3.05 a	35.67 a	5.22 a
AM1 (indigenous mycorrhizal mixture)	53.66 a	22.14 b	2.60 b	30.10 b	5.33 a
AM2 (*Glomus intraradices*)	42.27 b	19.74 c	2.03 c	27.11 c	5.00 a

-Values followed by the same letter are not significantly (P<0.05) different according to Fischer's LSD test.

Table III. Effect of mycorrhizal inoculation and NP fertilizer application on macronutrient (N & P) contents of onion plants

Treatment	Macronutrient content (%)	
	N	P
Control	2.37 b	0.14 b
NP fertilizers	3.80 a	0.28 a
AM1 (indigenous mycorrhizal mixture)	3.71 a	0.29 a
AM2 (*Glomus intraradices*)	2.56 b	0.28 a

-Values followed by the same letter are not significantly (P<0.05) different according to Fischer's LSD test

increase in fresh and dry weights above control, respectively. AM1 plants came second with 35 and 46% increase, followed by AM2 plants, which had 20 and 14% increase in fresh and dry weights, respectively. Unlike AM2, the increased biomass of AM1 and NP plants was reflected in their height being 29 and 32% taller than control plants, respectively. The number of leaves per plant was un-affected by any treatment.

Plant content of N, P and selected micronutrients. AM1 and AM2 fungi as well as NP fertilizers all produced significant increase in shoot P content (Table III). The P value for these treatments was more or less equal ranging between 0.28 to 0.29% i.e., 100% increase above control. N content, however, increased significantly only in AM1 and NP plants (Table III). In these plants, N level was in the range of 3.71 to 3.80% with a 56-60% increase above control.

The micronutrients contents of the various treatments are presented in Table IV. Shoot Zn increased significantly

Table IV. Effect of mycorrhizal inoculation and NP fertilizer application on the content of selected micronutrients of onion plants

Treatment	Micronutrients content (mg kg^{-1})				
	Zn	Cd	Fe	Cu	Mn
Control	7.42 b	0.23 b	205.00 c	3.43 a	20.23 a
NP fertilizers	7.57 b	0.15 b	253.33 b	3.97 a	20.58 a
AM1 (indigenous mycorrhizal mixture)	10.25 a	0.38 a	281.67 a	3.42 a	20.92 a
AM2 (*Glomus intraradices*)	10.23 a	0.15 b	245.00 b	3.70 a	18.58 a

-Values followed by the same letter are not significantly (P<0.05) different according to Fischer's LSD test

Table V. Selected properties of the soils used in the various treatments at the end of the experiment

Treatment	pH	EC (dS m^{-1})	P (mg kg^{-1})
Control	8.06 a	0.51 a	7.62 c
NP fertilizers	8.06 a	0.59 a	13.82 a
AM 1 (indigenous mycorrhizal mixture)	8.10 a	0.66 a	9.93 b
AM 2 (*Glomus intraradices*)	8.13 a	0.61 a	9.33 b

-Values followed by the same letter are not significantly (P<0.05) different according to Fischer's LSD test

in response to AM1 and AM2 inoculation, but not to NP fertilization. In both AM1 and AM2 plants, Zn level was about 10 ppm with an increase of 35 and 38% above NP-treated and control plants, respectively. AM1 produced the highest Fe level with 37% increase above control. AM2 and NP plants had similar Fe values with an increase of only 20 - 24%. In addition, only AM1 inoculation, significantly elevated Cd content resulting in 65% increase above all other treatments. No effect was produced by any treatment on Cu and Mn contents (Table IV).

Soil analysis after harvest. By comparing the soil before and after the experiment, there was no change in pH or electrical conductivity, but elevated P levels in all test soils (Table V). The increase of P was significantly higher in NP treated soil being about 80% above the control soil. In AM1 and AM2 inoculated soils, on the other hand, P levels were relatively equal, but much lower i.e., with an increase of only 30 and 22%, respectively.

DISCUSSION

Results of this study suggest that the growth of onion plants was enhanced significantly by inoculation with both types of AM fungi tested; the indigenous mixture (AM1) and the commercial *G. intraradices* (AM2); albeit to a lesser extent than in NP treated plants. Moreover, the growth extent in these AM1 and AM2 inoculated plants positively correlated with the rate of AM root colonization being higher in the former plants. This higher colonization rate achieved by AM1, might indicate adaptation of these indigenous fungi to their native soils in the sense of benefiting their hosts best under these given soil conditions. An increasing volume of research confirms that AM fungi differ in their ability to inoculate particular plant species and that AM fungi tend to exhibit the highest response in their

soils of origin (Bohrer *et al.*, 2003; Klironomos *et al*., 2003).

Results also indicated a small percentage of AM infection in uninoculated and NP-treated plants (18 & 24%, respectively) implying that contamination might have occurred at some stage during the experiment. Stottlemyer *et al.* (2008) indicated that aerial mycorrhizal spores are a potential source of contamination in mycorrhizal studies that are performed in greenhouses. Consequently, they suggested conducting such experiments inside air-filtered growth chambers to reduce the level of fungal contaminants. Other sources of contamination are also possible. For instance, the growing mix of seedlings, or may be that fumigation of the test soil with methyl bromide only once may have not been enough to kill all indigenous mycorrhizal propagules.

In this study, the highest growth rate was observed for NP treated plants implying that NP fertilizer has a more positive effect on the growth of onion plants than AM fungi. Yet, it could be that in our case, AM inoculation was performed at a rather late stage i.e., seedling stage and thus the performance of these fungi could have been improved had inoculation been done earlier at seeding time. Nevertheless, even if a slight reduction in crop yield is the outcome of forsaking chemical fertilizers, AM use still provides a better choice since it presents a sustainable and environmentally safer option.

In this experiment, P content in AM1 and AM2-inoculated plants and in NP-treated plants was equivalent being 100% higher than in control plants. This indicates that AM1 and AM2 fungi have the same ability to deliver P to onion plants and to a level equal to applying 30 mg P fertilizer kg^{-1} soil. The increased P uptake in AM inoculated plants is well documented (Smith & Read, 1997; Clark & Zeto, 2000; Javaid, 2009). It has been attributed to the extended AM hyphae, which explore a larger volume of soil and to P solubilization by AM root exudates from un-available sources present in the soil. Our data also indicated that NP, AM1 and AM2 plants, which had a similar P content (0.28%), produced variable levels of plant growth (1.7, 1.5 & 1.1 times greater than control plants, respectively) indicating no correlation between the two parameters. The effects of AM P uptake on host plants are poorly documented and available studies are not in agreement. Nonetheless, the lesser growth observed in AM plants compared to NP plants could be due to the fact that maintaining AM associations in the roots has a cost in translocation of sugars to the root system and that could translate into reduced growth (Douds *et al.*, 1988).

Unlike P, the N uptake of the various treatments was variable and higher in AM1 than in AM2 plants. The latter having an N content not significantly different from control. It has been suggested that AM acquire N from organic substances by enhancing decomposition (Hodge *et al.*, 2001). Therefore, it can be postulated that AM1 fungi might have been better decomposers of organic substances. Alternatively, the increased plant N in AM1 plants could be due to the higher quantity of AM1 fungi present on roots.

The ability of AM fungi to make immobile elements such as Zn and Cu more available to plants in general is well documented. In onion plants, higher Zn as a result of AM inoculation was also reported (Mahaveer & Alok, 2000; Ward *et al.*, 2001). In this study, Zn as well as, Fe uptake was improved in both AM1 and AM2 plants, while Cd uptake was increased in only AM1 plants. Except for Fe, plants grown with added NP fertilizer showed reduced plant uptake of all the tested trace elements. The precise mechanisms responsible for these observations are un-clear. If anything, these results support the concept that plant host-AM-soil environment interactions are extremely complex as well as, variable.

Other researches have indicated that AM inoculation tends to decrease pH in the rhizosphere soil, but not in the bulk soil (Mohammad *et al.*, 2005). This pH decrease was attributed to the ability of AM plants to produce more CO_2, which eventually leads to increase in soil acidity. Our study, however, indicated no change in soil pH by any of the treatments at the end of the experiment. This is most likely due to the high buffer capacity, which typifies our calcareous soil. Contrary to soil pH, a significant increase in P content of AM1 and AM2 soils was attained. This is clearly a result of P solubilization by AM root exudates from soil organic or inorganic phosphates.

CONCLUSION

Mycorrhizal inoculation tended to increase macro- and micronutrient and increased growth of onion, which was comparable to NP fertilizer application. Here, the benefits obtained from indigenous AM fungi surpassed those of commercial types. Thus, use of AM fungi economize on fertilizer use in plant production providing a sustainable and environmentally safer substitute. Further research is imperative for field appraisal of these fungi.

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