INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
16–920/2017/19–2–341–347
DOI: 10.17957/IJAB/15.0287
http://www.fspublishers.org

Full Length Article

Effects of Arbuscular Mycorrhizal Fungi and Fertilization Levels on Industrial Tomato Growth and Production

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Abstract

The effect of arbuscular mycorrhizal fungi (AMF) on industrial tomato (*Lycopersicon esculentum* Mill. cv. Isma F1) cultivated in North-Eastern Algeria was evaluated under greenhouse and field conditions in a vertisol soil intended for the cultivation of industrial tomato. In the greenhouse, a commercial AMF inoculum and native fungal isolates consisting of *Funneliformis mosseae* and *Septoglomus constrictum*, were added in sterilized or non-sterilized soil and tested for their effects on the growth of tomato. Inoculation with exotic AMF in the commercial inoculum or with native isolates had similar effects in increasing mycorrhizal root colonization rates and tomato growth. In the field, the commercial AMF inoculant was combined with three rates of chemical fertilizer used for the cultivation of industrial tomato (mono-ammonium phosphate, ammonium sulphate and NPK, applied at 0, 50 and 100% of the recommended dose). Introduced AMF significantly improved mycorrhizal root colonization levels, growth and yield as compared to the indigenous AMF alone. In addition, in the presence of the mycorrhizal inoculant, an application of 50% of recommended fertilizer dose provided the same yield as the full fertilizer dose without inoculation. Results clearly show that in this soil, plants needed both fertilization and AMF inoculation to achieve optimal growth and yield, and that the application of AMF can compensate for the reduction in chemical fertilizers, offering a more sustainable farming system that is respectful of the environment.

Keywords: *Funneliformis mosseae*; *Septoglomus constrictum*; Commercial AMF inoculum; Industrial tomato; Yield

Introduction

Many agricultural soils in the world are impoverished in one or more of the essential nutrients required for plant growth and yield so that fertilizer additions are essential to ensure maximum yields (Baligar *et al*., 2001). However, harmful quantities of fertilizers are applied annually in terrestrial agrosystems because they are not absorbed effectively (Baligar *et al*., 2001; Malhi *et al*., 2002), and fertilizer rates used are destined to increase in the future if the same conventional practices are applied to increase food production to satisfy the increasing world population (Tilman *et al*., 2002; Cordell *et al*., 2009). In addition, phosphorus in phosphate fertilizers comes from non-renewable resources and does not have any substitute for the growth of plants (Van Vuuren *et al*., 2010).

It is therefore important to optimize the efficiency of fertilizer use in agricultural production (White and Brown, 2010). This can be achieved by a more effective action of plants in the absorption and utilization of nutrients. In this context, symbiotic arbuscular mycorrhizal fungi (AMF) are an essential component to consider (Gianinazzi *et al*., 2010). These fungi are associated with roots and promote plant growth, increasing yields (Waller *et al*., 2005) by enhancing the absorption of available phosphorus from soil and of other poorly mobile mineral nutrients essential for the growth of plants (Clark and Zeto, 2000; Smith and Read, 2008). Their effectiveness in improving nutrient acquisition results mainly through extraradical mycelium exploring soil and transferring nutrients to the plant, so expanding the root absorption zone at distances up to several centimeters from the root (Smith and Read, 2008). The assistance of these microorganisms is especially valuable when nutrients are more difficult to absorb because they are "fixed" in the soil. They also limit filtration and nutrient loss to the environment, and so contribute to an integrated nutrient management (Adesemoye *et al*., 2008).

AMF have been shown to improve the growth and yield of many crop plants, especially in soils with poor to medium contents of available nutrients (Smith and Read, 2008). Tomato is particularly responsive to AMF inoculation, and previous work has shown effects on plant growth, nutrient contents or yield also in combination with NPK fertilizer or different P levels (Poulton *et al*., 2002;

To cite this paper: Ziane, H., A. Meddad-Hamza, A. Beddiar and S. Gianinazzi, 2017. Effects of arbuscular mycorrhizal fungi and fertilization levels on industrial tomato growth and production. *Int. J. Agric. Biol.*, 19: 341–347

Mujica *et al*., 2010; Conversa *et al*., 2013). However, the combination of AMF with a full fertilizer program for industrial tomato production in the field has not yet been evaluated.

The present work was carried out on tomato for industrial use, in North-Eastern Algeria. Industrial crops are a principal vocation in this region, mainly the cultivation of tomato which has known a very important expansion (FAO, 2005; Bedrani, 2008). Algeria uses lower amounts of fertilizers compared to the global average (FAO, 2005). In spite of this, the use of fertilizers in this region has had a negative impact on the environment including the pollution of surface water (Derradji *et al*., 2007; Harrat and Achour, 2010). Consequently, the objective of this work was to assess the mycorrhizal potential in promoting a more productive agriculture and less dependent on chemical fertilizers. Thus, a first step was to determine the efficiency of a commercial AMF inoculum and of native AMF strains isolated from an Algerian soil on the growth of tomato plants, under greenhouse conditions, in a soil intended for the cultivation of industrial tomato. Then, a study was conducted in a similar soil under field conditions where the same commercial inoculum was used in combination with different rates of chemical fertilizers on the growth, yield and number of fruits of industrial tomato. The aim was to evaluate the balance between bio-fertilization based on mycorrhizal fungi and chemical fertilization in order to ensure good fruit production in a system respectful of the environment.

Material and Methods

Greenhouse Experiment

The experiment was conducted in the greenhouse of the Badji Mokhtar University located in the commune of Sidi Amar, Annaba province, under natural daylight of 14 hours, a daily average temperature of 20-25°C and relative humidity of 60-70%. A clay soil, classified as a vertisol, was collected from a farm field in El Kerma, commune of El Hadjar, Annaba province (36° 44’ 35’’N; 7° 39’ 59’’E). The soil physico-chemical properties were determined in the agronomic laboratory of the fertilizer company FERTIAL (Annaba, Algeria) as: 28% sand, 44% clay, 28% silt; pH 7.85 ($H2O$); 20.60 ppm available P; 0.78% total C; 0.58 meq/100g K; 41.77 meq/100g Ca; 5.35 meq/100g Mg; 0.50 meq/100g Na; 1.33% organic matter. A portion of the soil sample was sterilized (SS) twice in an autoclave, 20 min at 120°C with 24 h between each autoclave, and the rest was not sterilized (NS).

The natural richness of the field soil in indigenous AMF propagules was assessed using the most probable number (MPN) method (Porter, 1979), which consists in making a series of successive soil dilutions and determining the limiting dilution at which no AMF propagules can be detected. For this, the collected soil was mixed with sterilized soil to make five serial dilutions (1/10) and five repetitions per level of dilution in 50 mL pots; controls contained sterile soil. One pre-germinated clover seed was planted into each pot. Plants were grown in the greenhouse (average temperature 18-22°C, relative humidity 60-70%, daylight of 14 h) and watered with distilled water. After six weeks, the entire root system per plant was harvested and stained according to the method of Philips and Hayman (1970) using acid lactic. Mycorrhizal or non-mycorrhizal roots were recorded for the 5 repetitions at each dilution level and the MPN of propagules per 50 g of soil was estimated according to Cochran (1950).

Tomato plants of a commercial variety (*Lycopersicon esculentum* Mill. cv. Isma F1) were produced in a private nursery, located in Ben Amar, commune of El Chatt, El Tarf province, Algeria. Seeds were germinated in boxes filled with peat, watered and placed in a germination chamber for 72 h, then in a greenhouse for 7 days and finally transferred into an acclimatization room for 10 days. This tomato variety has a 120 day cycle, and produces fruits with a very bright red color, of elongated shape, a good caliber, a 6° brix sugar content, pH 4.3 and an average weight of 100 g.

The commercial inoculum used, Symbivit® (INOCULUM *plus*, France), is granulated and consists of a support based on natural clay and propagules of 6 AMF species (*Claroideoglomus etunicatum*, *C. claroideum*, *Glomus microaggregatum*, *Rhizophagus intraradices*, *Funneliformis mosseae*, *F. geosporum*). The native fungal isolates used consisted of spores of the AMF species *F. mosseae* and *Septoglomus constrictum*, extracted from a soil cultivated with olive tree *Olea europaea* L., located in the commune of Bekkouche Lakhdar (36° 42' 23"N, 7° 17' 49"E), Skikda province, Algeria (Meddad-Hamza *et al.*, 2010). The collected spores were multiplied on clover plants (*Trifolium pretense* L.) grown in a sterilized soil from the Experimental Field of the Technopôle Agroenvironnement, AgrOnov, 2110 Bretenière France. Newly formed spores were isolated by wet sieving according to the method of Gerdemann and Nicholson (1963) and used as inoculum. The experimental setup was simple randomization with 6 treatments repeated 7 times for a total of 42 plants. Inoculation was done at transplanting of the three week old tomato seedlings, in pots containing 3 kg of soil: 10 g (200 propagules) per plant of commercial inoculum (CI) or 120 spores per plant (60 spores per species) of the native fungal isolates (FI) were deposited near the roots in both sterilized (SS) and non-sterilized (NS) soil. Non-inoculated controls grown on SS and NS were established. The plants were maintained under greenhouse conditions (see above) for a period of 2 months and watered daily with sterile water.

After two months growth, the level of mycorrhizal root colonization, plant height, shoot and root fresh and dry weight (after drying at 70°C for three days) of the tomato plants were measured. To estimate mycorrhizal colonization (M%: intensity of mycorrhizal colonization in the root system), root samples from the seven plants per treatment were stained according to the technique of Phillips and

Hayman (1970) using acid lactic. Root observations were done on 5 repetitions of 30 root fragments of 1 cm, placed between slide and cover slip in a drop of glycerol. Annotation was made according to the method described by Trouvelot *et al.* (1986), using the software MYCOCALC (http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

Field Experiment

The experiment was conducted in the farm field cited above, from March 26, 2013 to July 30, 2013. The climate of this region is typically Mediterranean. During the cropping period, the minimum and maximum average temperatures were 12.4°C and 23.8°C, respectively; total precipitation was 266 mm. The field was cultivated with wheat before tomato. The physico-chemical properties of the soil, tomato plant production and the commercial AMF inoculum used are described above. The fertilizers usually applied by farmers to ensure good tomato yields in the field were: mono-ammonium phosphate (MAP) (12% NH_4, 52% P_2O_5), ammonium sulphate (21% NH_4, 60% SO_4) and NPK (8.10.30+35) (8% NH_4, 10% P_2O_5, 30% K_2 0, 35% SO_4), applied each at the recommended doses of 200 kg ha^{-1}, 200 kg ha^{-1} and 600 kg ha^{-1}, respectively.

The experiment was in a complete randomized block design consisting of three blocks representing three repetitions, each one subdivided into six plots, for a total of 18 plots. Each block contained all treatments and the distribution of treatments within the same block was made randomly. Each plot consisted of 4 lines spaced 1.20 m and six rows with a spacing of 30 cm between plants, with a buffering border of 2 m around each plot. Each plot had an area of 41.8 m² (7.6 m wide and 5.5 m long). Half of the plots received the commercial mycorrhizal inoculum (I) and the other half represented controls (NI). Three week old tomato seedlings were inoculated at transplanting by placing 10g inoculum per plant on the roots and immediately covering. The chemical fertilizers were applied at 0%, 50% and 100% of the recommended doses. This gave 6 experimental treatments: Non-inoculated (NI) + 0% chemical fertilizers (CF), Inoculated (I) + 0% CF, NI + 50% CF, I + 50% CF, NI+ 100% CF and I + 100% CF.

After three months cultivation, 7 plants in each plot were randomly selected for estimating the mycorrhizal root colonization, plant height, and shoot and root dry weights. At harvest, the average number of fruits per plant and overall fruit yield (t ha^{-1}) were estimated. Percentage growth and yield increases were calculated using the following formula (Plenchette *et al.,* 1983):

Growth increase (%) = [(growth of inoculated plants - growth of non-inoculated plants)/growth of inoculated plants)] × 100.

Yield increase (%) = [(yield of inoculated plants - yield of non-inoculated plants)/yield of inoculated plants)] × 100.

Statistical Analysis

For both experiments, an analysis of variance (ANOVA) with general linear model (GLM) was used to determine the effect of factors in the software MINITAB 16. Comparisons between means were performed with Tukey's test Honestly Significant Difference (HSD) at the significance level $P<0.05$.

Results

Greenhouse Experiment

AMF soil potential and mycorrhizal root colonization: The number of infective propagules of indigenous AMF (MPN) in the field soil before the start of the experiment was 45 ± 0.41 per 50 g of soil. Plants grown in NS had a significantly higher mycorrhizal colonization level compared to plants grown in SS, and the inoculation with CI or FI AMF inocula had an important effect on the mycorrhizal colonization of the tomato roots **(**Fig. 1). Plants inoculated by CI had a significantly higher average colonization level (51.7%) than plants inoculated by FI (44.5%).

Plant Growth

Inoculation of CI and FI had a significant impact on most of the tomato plant growth parameters measured (Table 1). Overall, inoculated plants had better growth compared to the non-inoculated ones: + 22% for height, + 23% for shoot fresh weight, + 16% for root fresh weight, and + 15% for shoot dry weight. No significant difference was observed between CI and FI treatments for any of the measured parameters. Plants grown in NS were significantly higher (+ 21%), had a greater shoot and root fresh weight (+21% and +10%) and a greater shoot dry weight (+ 10%) than those grown in SS. The interaction 'inoculation x soil treatment' was significant (Table 1).

Field Experiment

Mycorrhizal root colonization: Mycorrhizal colonization was observed in plants, both inoculated and non-inoculated with a commercial AMF inoculum, whatever the fertilization level (Fig. 2). However, the overall percentage of mycorrhizal colonization was significantly higher in the inoculated plants (40%) compared to non-inoculated plants (27%), especially in the treatment I + 0% CF (44.1%). Fertilization had a significant negative impact on root colonization in the non-inoculated plants and the lowest level occurred with 100% CF (26%), whereas this effect of the fertilizers was not significant in the inoculated plants (Fig. 2).

Table 1: Height, shoot and root fresh and dry weights of tomato plants inoculated with a commercial AMF inoculum (CI) or native fungal isolates (FI) and grown in the greenhouse

Growth parameters	Treatments							Significance 'Inoculation x soil treatment'
	Inoculation				Soil treatment			
	CI	FI	NI	Significance	SS	NS	Significance	
Height (cm plant^{-1})	39.9 A	40.2 A	31.2 B	P<0.05	32.6 B	41.5 A	P<0.05	P=0.118
Shoot fresh weight (g plant^{-1})	21.3 A	20.9 A	16.1 B	P<0.05	17.1 B	21.7 A	P<0.05	P<0.05
Root fresh weight (g plant^{-1})	5.8 A	5.7 A	4.8 B	P<0.05	5.1 B	5.7 A	P<0.05	P=0.151
Shoot dry weight (g plant^{-1})	8.7 A	8.5 A	7.3 B	P<0.05	7.7 B	8.6 A	P<0.05	P=0.938
Root dry weight (g plant^{-1})	1.4 A	1.4 A	1.3 A	P=0.105	1.3 A	1.4 A	P=0.072	P=0.997

Means in lines followed by different letters are significantly different (P <0.05) according to Tukey's HSD test

Table 2: Height, shoot and root dry weight of tomato plants inoculated with a commercial AMF inoculum and grown in the field with three levels of chemical fertilizers (0, 50 and 100% of the recommended doses)

Growth parameters	Treatments							Significance 'Inoculation x Fertilization'
	Inoculation			Chemical fertilization				
	I	NI	Significance	0%	50%	100%	Significance	
Height (cm plant^{-1})	48.4 A	39.7 B	P<0.05	40.9 C	43.2 B	48.1 A	P<0.05	P=0.115
Shoot dry weight (g plant^{-1})	89.8 A	51.7 B	P<0.05	59.6 B	75.4 A	77.0 A	P<0.05	P<0.05
Root dry weight (g plant^{-1})	9.7 A	7.8 B	P<0.05	7.2 C	10.6 A	8.5 B	P<0.05	P<0.05

Means followed by different letters are significantly different (P <0.05) according to Tukey's HSD test

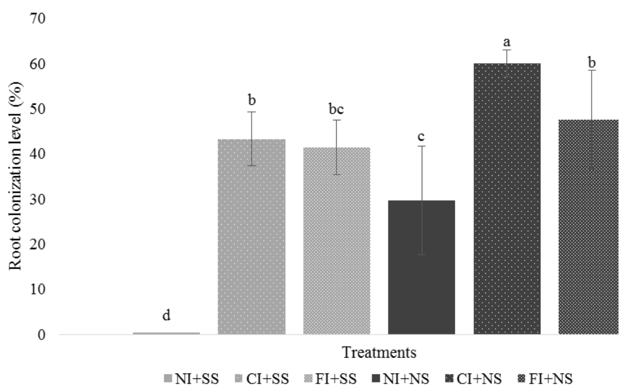

Fig. 1: Effect of a commercial AMF inoculum (CI) and native fungal isolates (FI) on mycorrhizal root colonization levels of tomato plants grown in the greenhouse in sterilized (SS) and non-sterilized (NS) soil, and compared to the controls (NI)

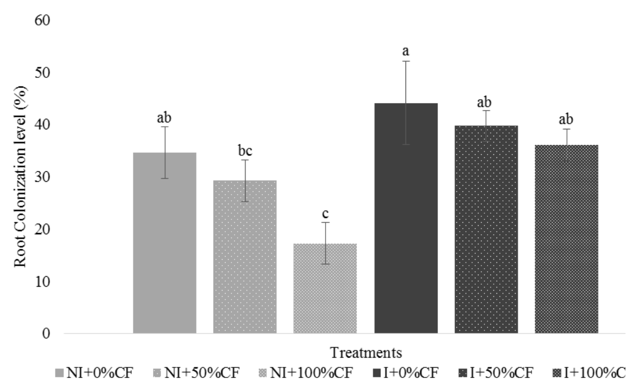

Fig. 2: Effect of chemical fertilizers (CF) on mycorrhizal root colonization of tomato plants inoculated (I) and non-inoculated (NI) with a commercial AMF inoculum

Plant Growth

Regardless of the level of chemical fertilization, the overall height of tomato plants inoculated with the commercial AMF inoculum was significantly greater (48.4 cm plant^{-1}) compared to non-inoculated plants (39.7 cm plant^{-1}), representing a gain of 18%. Fertilization increased plant height which was greater by 15% with the dose 100% CF compared to 0% CF. Shoot and root dry weights were also significantly greater in inoculated plants compared to non-inoculated plants (+42% and + 19%, respectively). Again, fertilization had a significant impact on shoot dry weight, with an increase of 22% between the doses 100% and 0% CF, and resulted in a significant increase in root dry weight (+32%) between the doses 50% and 0% CF. The interaction inoculation× fertilization was significant for both shoot and root dry weight (Table 2).

Yield and Number of Fruits

Mycorrhizal inoculation had a significant impact on the overall yield of tomato plants, which was greater in inoculated plants (57.1 t ha^{-1}) compared to non-inoculated plants (43.93 t ha^{-1}) (+23%) (Fig. 3). An increasing level of fertilization also significantly increased fruit yield, and a further gain in total weight was observed of 21%, 24% and 22% with AMF inoculation in the treatments 0, 50 and 100% CF, respectively. It is noteworthy that no significant difference was observed between the yield obtained from the inoculated plants receiving 50% CF and the non-inoculated plants receiving 100% CF (Fig. 3). The number of fruits consistently increased, but not significantly, in

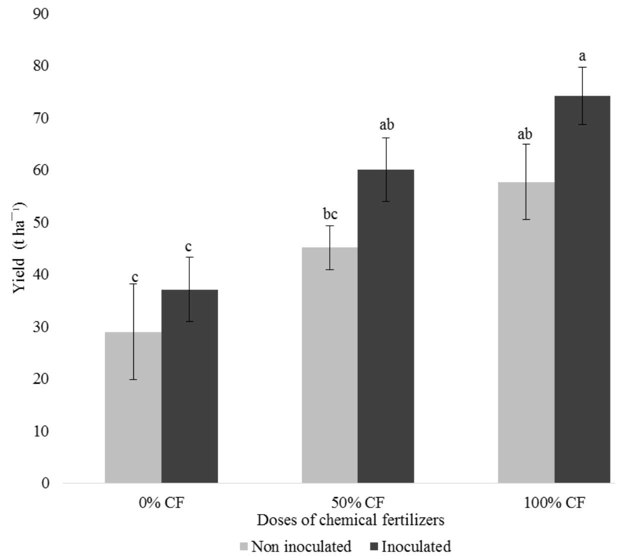

Fig. 3: Yield of tomato plants inoculated with a commercial AMF inoculum and grown in the field with three levels of chemical fertilizers (0, 50 and 100% of the recommended doses)

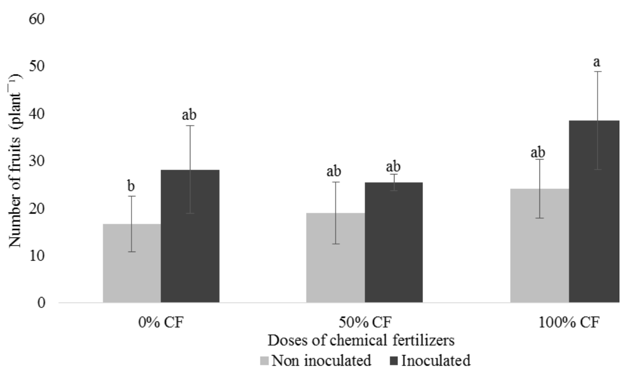

Fig. 4: Number of fruits of tomato plants inoculated with a commercial AMF inoculum and grown in the field with three levels of chemical fertilizers (0, 50 and 100% of the recommended doses)

AMF-inoculated tomato plants compared to non-inoculated plants, with an average of 30.6 and 19.9 fruit plant^{-1}, respectively (+35%). Fertilization had no significant impact on the number of fruits. Nevertheless, increases of 41%, 25% and 37% were observed between inoculated and non-inoculated plants in the treatments 0, 50 and 100% CF, respectively (Fig. 4).

Discussion

In the present study on the production of industrial tomato in an Algerian vertisol field soil, growth of tomato plants in the greenhouse was most pronounced in the non-sterile soil, which also promoted the highest mycorrhizal root colonization levels. These were further increased with the commercial AMF inoculum and the native fungal isolates, which probably reflects the development of a more important network of hyphae in the mycorrhizosphere of the inoculated plants. The results are in agreement with those of Zhong-Qun *et al.* (2007) and Tahat *et al.* (2008) who showed that the root colonization levels were positively correlated with the growth of tomato. Furthermore, the inoculation of several AMF probably increased the number of AMF species in the non-sterile soil. AMF species richness and interaction have been reported to have a positive impact on plant growth (Pellegrino *et al*., 2011; Ortas and Ustuner, 2014), and a functional complementarity has been suggested between species within the AMF community colonizing a single root system (Jansa *et al.*, 2008). The field soil used in the present study is moderately poor in infective AMF propagules (900 per kg of soil), if compared to other studies (Gianinazzi-Pearson *et al.*, 1985; Maiti *et al.*, 2011), and is therefore amenable to the addition of exogenous inoculum to promote root colonization by AMF. The native fungal isolates and the commercial AMF inoculum were not significantly different in stimulating the growth of tomato. Ortas and Ustuner (2014) also showed that inoculation of *Citrus aurantium* L. with native spores had the same effect as a commercial inoculum, and Pellegrino *et al.* (2011) reported that native mycorrhizal inoculum was as effective on *Trifolium alexandrinum* L., or even more, than exotic fungal isolates, whilst Schreiner (2007) showed that native AMF isolates are not necessarily better adapted to specific soils in improving growth and nutrient absorption.

As may be expected, the roots of tomato plants were colonized by AMF in all treatments in the field experiment. However, the colonization of inoculated plants was significantly higher than that of non-inoculated plants. This may result from the introduced AMF being more effective in root colonization than the indigenous AMF alone, corroborating the results of Tawaraya *et al.* (2012) on *Allium fistulosum* L., but it may also be directly related to introduced inoculum enriching the mycorrhizal potential of the field soil. Mycorrhizal root colonization of non-inoculated plants decreased with the increasing dose of chemical fertilizers whilst, interestingly, the colonization levels of the inoculated plants did not change significantly. Although it is known that increasing P fertilization in the soil can reduce AMF root colonization and spore density (Al-Karaki, 2013), Ortas (2012) suggested that when increased levels of P are used, these may not be high enough to inhibit the colonization by some AMF species, which could be the case with the commercial inoculum used in our experiments.

AMF inoculation in the field had a positive effect on all tomato growth parameters compared to non-inoculated plants, suggesting that the introduced AMF may be more effective than the indigenous AMF, as already observed by

Ortas under long-term field conditions (Ortas, 2015). As the mycorrhizal soil potential indicated that the soil was moderately poor in infective propagules, indigenous species could be either scarce or inefficient because of soil disturbance caused by agricultural practices which tend to be harmful to AMF populations (Hamel, 1996; Gosling *et al.*, 2006).

Fertilization had a positive impact on the growth of tomato in the field, with a minimum of 50% CF being enough to give maximum shoot and dry weights. In the absence of fertilizers (0% CF), plants had a very poor growth despite the high mycorrhizal root colonization levels. This is probably due to the nutrient deficiency of the field soil which, according to standards of soil analysis interpretation (Soltner, 1981), had a very low P level, low K, medium Mg and very low organic matter content. Teste *et al.* (2016) reported that despite a strong root colonization by AMF and a great abundance and diversity of potential host plants, P deficient soils induce the lowest biomass of extraradical hyphae that allow increased acquisition of P and, thus, plant growth. This would explain why tomato plants needed both AMF inoculation and fertilization to achieve sufficient growth in the nutrient poor field soil used in the present study. Subhashini (2016) showed that in a soil poor in nutrients, the addition of NPK and AMF led to significant increases in plant biomass of *Nicotiana tabacum* L. On the other hand, Schreiner (2007) reported that grapevines grown in P-deficient soils are heavily dependent on AMF to acquire the necessary P for growth and acquisition of other nutrients, whilst grapevines grown in more fertile soils are less dependent on AMF although they still benefit from greater P absorption.

The highest tomato yields were obtained in the field with the commercial AMF inoculum plus 100% CF, which is in agreement with Mujica *et al.*'s (2010) observation that tomato yields increased after the application of AMF combined with a full dose of P fertilizer. Hu *et al.* (2010) also reported that the yield and P acquisition of wheat plants grown with a long-term NPK application were clearly limited but that they could be significantly increased by inoculation with the AMF *Glomus caledonium*. Taken together, these results suggest that the rational combination of AMF and NPK chemical fertilizer can be advantageous to crop production. In fact, the present study also shows that in the presence of the commercial AMF inoculum, an application of 50% of the recommended dose of the chemical fertilizers to field grown tomato plants provides the same yield of fruit as the full dose of fertilizers without inoculation; this treatment represents the best combination for an optimal yield. A similar result has been obtained in the cultivation of watermelon in North-Eastern Algeria, where the same commercial inoculum was used. These and previous studies (Poulton *et al.*, 2002; Tawaraya *et al.*, 2012; Zhang *et al.*, 2015) consolidate the idea that the application of AMF inoculation has the potential to reduce the use of chemical fertilizers in large quantities.

Conclusion

Inoculation of tomato with AMF had positive impacts on plant growth and yield in a non-sterile vertisol. Native fungal isolates could represent good candidates for producing indigenous inoculum, and the fact that tomato yield was maintained by combining AMF inoculation with a 50% reduction in the recommended chemical fertilizer dose, as compared to the full dose without inoculation, confirms that the application of AMF can be exploited as an environmentally friendly biotechnology in a more sustainable farming system.

Acknowledgements

We are grateful to Nouar Abdelli for allowing us to conduct experiments at his farm field while meeting our requirements and to Vivienne Gianinazzi-Pearson for her valuable comments and suggestions to improve this manuscript.

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(Received 31 December 2016; Accepted 26 January 2017)