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ORIGINAL PAPER

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APPLICATION OF ARBUSCULAR MYCORRHIZAL INOCULUM IN GREENHOUSE SOIL WITH MANURE INDUCED SALINITY FOR ORGANIC PEPPER PRODUCTION

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ABSTRACT

In this study, the effect of arbuscular mycorrhizal fungus − AMF *Rhizophagus intraradices* inoculum (prior or with transplanting) to different pepper type (*Capsicum annuum* L.) cv. Arlequin F1 (long fruits) and Raiko F1 (bell pepper), on plant growth and physiological parameters in response to elevated soil P concentrations from organic greenhouse production with enhanced soil salinity, was investigated. To explain the physiological growth of mycorrhizal inoculated (M) and non-mycorrhizal inoculated (NM) plants, the parameters of fungal root length colonization, shoot concentration of P an N during growth, plant height, width of stem, yield, number of fruit per plant and also the quality parameters of fruits such as soluble solid content (SSC), fruit color, mineral profile, total soluble phenolics (TSP) and antioxidant activity (FRAP), were determined. This study showed that application of AMF in cv. Raiko cultivated in high P saline soil generally enhanced growth, fruit yield and number of fruits per plant when inoculated at planting time in the greenhouse. AM inoculated plants, regardless of the time of application in cv. Arlequin grown under the same conditions, did not have any significant differences in comparison with NM plants. Arbuscular mycorrhizal inoculation has great potential in enhancing the pepper growth and yield even in high soil P, however, because of the complexity and interaction of involved genotypes of pepper and AMF, the method and time of inoculation, the system of pepper production and environmental conditions, as well as assays have to be performed to verify positive effects.

Key words: *Rhizophagus intraradices*, *Capsicum annuum*, manure, organic farming, color, SSC, phenols, antioxidants

INTRODUCTION

The arbuscular mycorrhizal fungi (AMF) create a ‘bridge’ between the soil and plant, taking up nutrients from the soil and delivering them to the plant, from which they intake carbohydrates and lipids produced in the process of photosynthesis from the plant [Bravo et al. 2017, Jamiołkowska et al. 2017]. Majority of vegetable crops are potential host plants of arbuscular mycorrhizal fungi. AMF increase the absorption surface of plant roots and the bioavailability of some nutrients and contribute to enhance

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nutrient and water uptake by plants [Baum et al. 2015]. Moreover, these fungi stimulate secretion of plant hormones [Bagyaraj et al. 2015], increase the intensity of photosynthesis [Zhu et al. 2014] and plant tolerance to abiotic and biotic stress factors, including heavy metals [Abdel Latef 2013], drought [Vincente-Sánchez et al. 2014], biological control of root pathogens [Bagyaraj et al. 2015] and salinity [Beltrano et al. 2013].

Local or native AMF inocula, defined respectively as fungi isolated from the same field or from a wider region, which shares common environmental conditions and agricultural practices, may be better adapted to local soil and environmental conditions, and may be more capable of competing with indigenous AMF community [Pellegrino et al. 2011]. Klironomos [2003] showed that there is a range of negative to positive interactions between particular AM fungus and different plants with combinations of local inoculum with local plants frequently showing extreme responses, highly positive or highly negative.

Mycorrhizal technology is being used more frequently in horticultural vegetable production and an international mycorrhizal industry is developing [Vosátka et al. 2008]. Therefore, inoculation of vegetable crops with AMF can be profitable [Baum et al. 2015] and commercial inoculation products like Symbivit [Hernádi et al. 2012], Mycoroot^{TM} [Maboko et al. 2013] and many others are available. However, mycorrhiza used in pepper crops (*Capsicum* spp.) is still rarely exploited compared to other crops of economic importance [Pereira et al. 2016]. Pre-inoculation of green pepper transplants with AM fungi have positive enhancement effects in reducing the effects of salt stress [Al-Karaki et al. 2017]. Mycorrhizal inoculation is capable of maintaining the membrane stability and growth of pepper plants under salt stress, and this could be related to P nutrition [Beltrano et al. 2013]. Inoculated pepper plants showed increased chlorophyll index and leaf contents of N, P, Fe and Zn, compared with non-inoculated plants [Diaz Franco et al. 2013]. Beneficial microbes and their combined (AMF + *Pseudomonas* + *Trichoderma*) inoculations have different potential to modulate defense enzymes and positively influence the pepper fruit yield under field conditions [Duc et al. 2017]. Tanwar et al. [2013] suggested that application of AMF (*G. mosseae* and *Acaulospora laevis*) with plant growth promoting bacteria (*Pseudomonas fluorescens*) along with 50% reduced doses of P fertilizer during seedling transplantation increased the overall growth and yield performance of pepper and could be considered as a sustainable substitute to high P fertilizer in pepper cultivation.

High P availability, usually occurring in high input agricultural systems, tends to suppress mycorrhizal symbiosis [Mäder et al. 2000]. In general, increasing the soil P concentrations decreases the mycorrhizal growth response of AMF [Smith and Smith 2011]. Even growth depression caused by AMF in non-host species or in host species are common when phosphate availability is high [Koide and Mosse 2004]. High P conditions may decrease the permeability of the root membrane that limits exudation and may retard the growth of AMF infection units and the rate of entry point formation, while the reduced benefit of mycorrhization on plant growth in high P soils may be the result of down-regulated root uptake [Smith et al. 2011]. However, in certain cases, active and effective symbiosis at high P soil levels has been observed [Douds et al. 2007]. This is often related to some stress that is active along with high P soil concentrations, such as drought, moisture and low temperature [Douds et al. 2012].

The main aim of this study was to explore the potential of AMF to enhance the nutrition, growth and yield of pepper plants (*Capsicum annuum* L.), an important crop, in a high P greenhouse saline soil with indigenous AM fungal community. Further, the effect of applying the AMF inoculum to pepper plants prior to transplanting in the greenhouse was explored as a possible method to enable the bioenhancing potential under high P and saline soil conditions.

MATERIALS AND METHODS

Experimental design. Two types of sweet pepper (*Capsicum annuum* L.) cultivars, Arlequin F_1 (long fruits) and Raiko F_1 (bell fruits), were used in commercial greenhouse production (plastic tunnels 3.5 m high, covered by 3-layer, long-life, thermic, EVA film − Kritifil 180μ, origin from Plastika Kritis, Greece, with following optical properties − total light transmission 89%; diffusion 45%; infrared transmission <17), located in Sapes, north-eastern Greece

(longitude: 25°42'E, latitude 41°01'N). In the local system, pepper plants are either grown from seed or purchased as young pepper plants and are then transplanted into their final positions in the greenhouse soil. Mean month temperatures in the area ranged from 5°C in January to 26.6°C in August (Tab. 1). The mean air temperature in the greenhouse ranged from 30 to 45°C. Mild weather conditions, low-level agrochemical pollution and small size family-farms promote the production of organic pepper in Sapes region with good organoleptic properties.

Seed was sown on February 23, 2016 in trays. At the second true-leaf stage, pepper plants were transplanted on March 26 to plastic pots (volume 0.2 L) containing a substrate consisting of 50% greenhouse soil, 30% goat manure and 20% peat (v/v) and a small part of marble dust. These plants were later transplanted again from the pots to their final positions in the greenhouse soil without removing the potting substrate at the stage of eight leaves (16–20 cm). Thirty experimental replicates were prepared for each treatment (each pepper plant was in a separate pot) according to a randomized complete block design. Seedlings were transplanted on 10th of April into plastic greenhouse and arranged in double rows with a distance of 0.8 between strips and 0.6 m between the rows and 0.6 m between plants in the rows. At least 5 extra plants per treatment (inoculated or non-inoculated) were sampled at the day of transplantation to determine the levels of root colonization by AMF and plant growth parameters (height, number of leaves, shoot and root dry weight, root/shoot ratio).

A randomized complete block design with one way treatment classification was applied in the greenhouse, with six sequential plants on the row per each treatment and cultivar in the block and five blocks. The greenhouse soil (62% sand, 14% clay, 24% silt, pH 7.7, organic matter 9.8%) was saline and with high organic matter content due to previous generous annual goat manure amendments with 4.0 t ha^{-1} (N – 1.92%, P_2O_5 – 1.14%, K_2O – 2.05%) and high P content (Tabs. 2 and 3).

Table 1. Mean air temperature and total precipitation during the vegetation period (April to September 2016 from Sapes meteorological stations)

Year Month	Mean monthly air temperature (°C)	Mean daily temperature maximum for the month (°C)	Mean daily temperature minimum for the month (°C)	Precipitation amount (mm)
April	15.9	23.9	9.6	7.8
May	17.1	23.5	11.6	91.2
June	23.7	31.1	17.5	8.0
July	26.4	33.8	19.0	5.0
August	26.6	34.3	19.4	0.0
September	21.5	29.2	15.3	0.0

Table 2. EC, $CaCO_3$ and elemental nutrient concentrations of soil in organic pepper production

	EC (mS/cm)	$CaCO_3$ (%)	P	K	Mg	Ca	Fe (ppm)	Zn	Mn	Cu	B
Autumn	18.98	12.5	460	7.13	3.79	>2.00	47.07	17.9	18.17	0.64	13.06
Spring	8.48	5.9	390	1.70	1.93	>2.00	36.72	13.29	8.84	1.22	8.60

EC – soil electrical conductivity

Table 3. Fungal percentage of root length colonization in the rhizosphere of *Capsicum annum* growing in an organic greenhouse

Treatments of mycorrhiza	September	
Hybrid	Raiko	Arlequin
NM* (control)	0.09 a	0.26 a
M** at nursery	0.12 a	0.14 a
M** at greenhouse	0.09 a	0.09 a

*NM − non-mycorrhizal plants. **M − mycorrhizal plants. Values followed by the same letter do not significantly differ between the treatments. Statistical analysis was done separately for each hybrid

The experiments were set in conditions of sprinkler irrigation, without any additional fertilization during the growing season. The sprinkler irrigation system was operated periodically during the vegetation period to maintain the soil moisture above 70% of holding water capacity. No chemicals or fertilizers were applied, and the greenhouse was not heated during cultivation.

Pepper plants were first harvested on June 9th and harvests followed according to fruit maturation until September 17th. In total, 14 harvests were performed. At each harvest, the total number and weight of harvested pepper fruits per experimental unit (five consecutive plants on the row) were recorded. After the last harvest, root samples were taken from the middle space between two consecutive plants on the row and were bulked to one per block and treatment. The roots were washed free of soil, stained and AMF root colonization was estimated according to Sylvia et al. [1993] using the dissecting scope and spreading the stained roots in Petri dishes for observing the roots at the time of transplantation to the greenhouse, while plant roots were sampled after the last fruit harvest and at least 25 stained 2 cm root pieces were placed on slides and colonization was counted under a microscope according to McGonigle et al. [1990].

The organic production in the experimental greenhouse was certified by private bodies approved by the Ministry of Agriculture, under Regulation (EC) No 834/2007 and the Regulation No 889/2008.

Soil analysis. A composite soil sample for analyzing the soil properties was taken (0−30 cm) from five random positions before the experiment and the composite sample was analyzed in duplicate. Soil reaction was determined in a suspension with H_2O and 1 M KCl (ratio of 1 : 2.5, w/v), using a Metrel MA3657 pH-meter. The $CaCO_3$ content was determined volumetrically, using Scheibler's calcimeter (Hedas, Serbia). Total soil C and N contents were determined with a CHNS analyzer (Elementar Vario EL, Germany). After extraction with AL-solution (0.1 M ammonium lactate and 0.4 M acetic acid, pH 3.75, with a 1 : 20, w/v ratio of soil : solution), phosphorus concentration was measured with a spectrophotometer, and potassium concentration with a flame photometer.

Fungal material. The AMF inoculum was a *Rhizophagus intraradices* (formerly called *Glomus intraradices*), isolate that had been previously isolated from a certified organic vegetable farm located in northern Greece and identified *via* partial sequencing of small ribosomal subunit [Ipsilantis et al. 2012]. The inoculum was propagated with corn as a host, using an autoclaved 1 : 1 : 1 sand : vermiculite : perlite potting mix as a substrate, providing full strength Hoagland, but with 10% P, weekly. After at least 3 months of growth, the substrate was allowed to dry, the roots were cut and the inoculum (consisting of at least 550 spores in 100 g, root pieces, hyphae and substrate) was maintained at 4°C until use. The inoculum was applied by placing 30 g in the transplantation hole for field inoculated plants, or by mixing the inoculum at 10% rate (v/v) with soil : manure : peat (50 : 30 : 20) potting mix. Control plants received autoclaved inoculum potting medium instead and inoculum filtrate was not applied.

Fruit analysis. For the compositional analysis of pepper, two fruits of separated plants of each replication were harvested on 21st of July as well as 3rd September. Fruits of each replication were macerated in a blender (7011HS, Waring, Japan). Soluble solids content (SSC) was determined in the juice of blended material using an Atago PR-1 refractometer (Atago Co. Ltd., Tokyo, Japan). Total soluble phenol content was determined with the Folin–Ciocalteu reagent according to Scalbert et al. [1989].

Total antioxidant activity of fruits was determined by the use of FRAP assay according to Benzie and Strain [1999].

Statistical analysis. Data were subjected to one-way ANOVA for each hybrid separately to investi-

gate significant effects of inoculating with AMF on the variables measured. Means were compared with the Duncan's multiple range test at $\alpha = 0.05$. Mycorrhizal root colonization data were arcsin transformed before analysis. Pearson correlation coefficients between variables were calculated.

RESULTS

At the stage of transplanting the pepper plants from pots to the greenhouse soil, mean AM fungal colonization of roots of AM pre-inoculated seedlings was 1% with colonization still at initial stages, while no AM fungal colonization was observed in the roots of non-inoculated seedlings. At the end of the growing season, AM root length colonization was still low, ~10%, with no differences among any treatments (Tab. 3). There were positive correlations between AM fungal colonization and pepper fruit P content, for which there were also no differences among treatments, as there were none for N, neither (Tab. 4).

Table 4. Phosphorus (P) and nitrogen (N) concentration (%) in M and NM pepper during grown

Cultivars	P				N			
	July		September		July		September	
	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin
NM* (control)	1.40a	1.72a	1.07a	1.22a	22.47a	26.18a	18.60a	16.93a
M** at nursery	1.34a	1.67a	1.23a	1.42a	24.66a	26.69a	20.97a	21.11a
M** at greenhouse	1.31a	1.68a	1.18a	1.93a	23.10a	24.87a	20.14a	20.85a

*NM − non-mycorrhizal plants. **M − mycorrhizal plants. Values followed by the same letter do not significantly differ between the treatments. Statistical analysis was done separately for each hybrid

Table 5. Pepper plant height (cm) during grown depending on the application time of fungal inoculum

Cultivars	April		June		July		August	
	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin
NM* (control)	28.4a	14.2a	56.2a	35.5a	75.3a	56.2a	135.3a	106.0a
M** at nursery	27.6a	17.7a	50.2a	35.7a	61.3a	52.7a	116.8a	101.3a
M** at greenhouse	29.2a	16.7a	55.3a	39.3a	77.0a	57.5a	120.0a	98.8a

*NM − non-mycorrhizal plants. **M − mycorrhizal plants. Values followed by the same letter do not significantly differ between the treatments. Statistical analysis was done separately for each hybrid

Table 6. Width (cm) of the stem (just above the soil surface 1−2 cm) in M and NM pepper plants during grown period

Cultivars	April		June		July		August	
	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin
NM* (control)	4.88a	3.37a	8.42a	8.17a	10.53a	10.38a	13.48a	14.90a
M** at nursery	5.64a	4.20a	8.58a	7.65a	9.85a	10.11a	13.28a	14.12a
M**at greenhouse	5.20a	3.72a	9.07a	8.05a	11.78a	11.42a	14.55a	14.87a

*NM − non-mycorrhizal plants. **M − mycorrhizal plants. Values followed by the same letter do not significantly differ between the treatments. Statistical analysis was done separately for each hybrid

Mycorrhizal inoculation did not increase the plant height and stem diameter (Tabs. 5 and 6). The opposite trend was observed for both cultivars in August. Stem width in all plants gradually grew, becoming solid and stable, without any differences among treatments or plant varieties (Tab. 6).

Pepper seedlings of cv. Raiko inoculated at the planting time in the greenhouse obtained the highest fresh yield (2.71 kg plant^{-1}) than those inoculated at nursery stage (1.88 kg plant^{-1}) and non-inoculated control plants (1.65 kg plant^{-1}). For cv. Arlequin, all treatments had similar fruit yield per plant (Tab. 7). The number of fruits per AM inoculated plant at planting time in greenhouse was significantly higher (31.2 ±0.7) in cv. Raiko than in AM inoculated plants at nursery stage (22.5 ±1.8) and non-inoculated control plants (19.2 ±1.5). Differences in fruit number per plant between treatments (time of inoculation) and non-inoculated plants in cv. Arlequin were not observed (Tab. 7).

No significant differences in color development of pepper fruit cv. Arlequin between M and NM plants were found. The color parameters (L*) of pepper fruit cv. Raiko in July were similar in both AM treatments (time of inoculation) and much higher than that of non-inoculated control plants (Tab. 8). At the end of the growing season in September, these differences in color development disappeared. Inoculation time for both cultivars had no effect on other color parameters (Hue and Chroma), in comparison with control non-inoculated fruit (NM) (Tab. 8).

We have found no differences among treatments and pepper cultivars in soluble solid content, total phenolic content and antioxidant activity. The TSP content in cv. Arlequin from M and NM plants during September was significantly higher compared to the content in fruit during July. Peppers harvested at different times could differ in their antioxidant activity. The cv. Raiko generally had stronger antioxidant activities than cv. Arlequin within the inoculated and non-inoculated plants in July, but at harvest in September, no difference among cultivars was observed. Concurrently, there was no difference in antioxidant capacity between inoculated and non-inoculated plants. Our results also show that the antioxidant activities were not significantly different between the two times of AM application (Fig. 1).

Table 7. Yield and parameters of yield in M and NM pepper

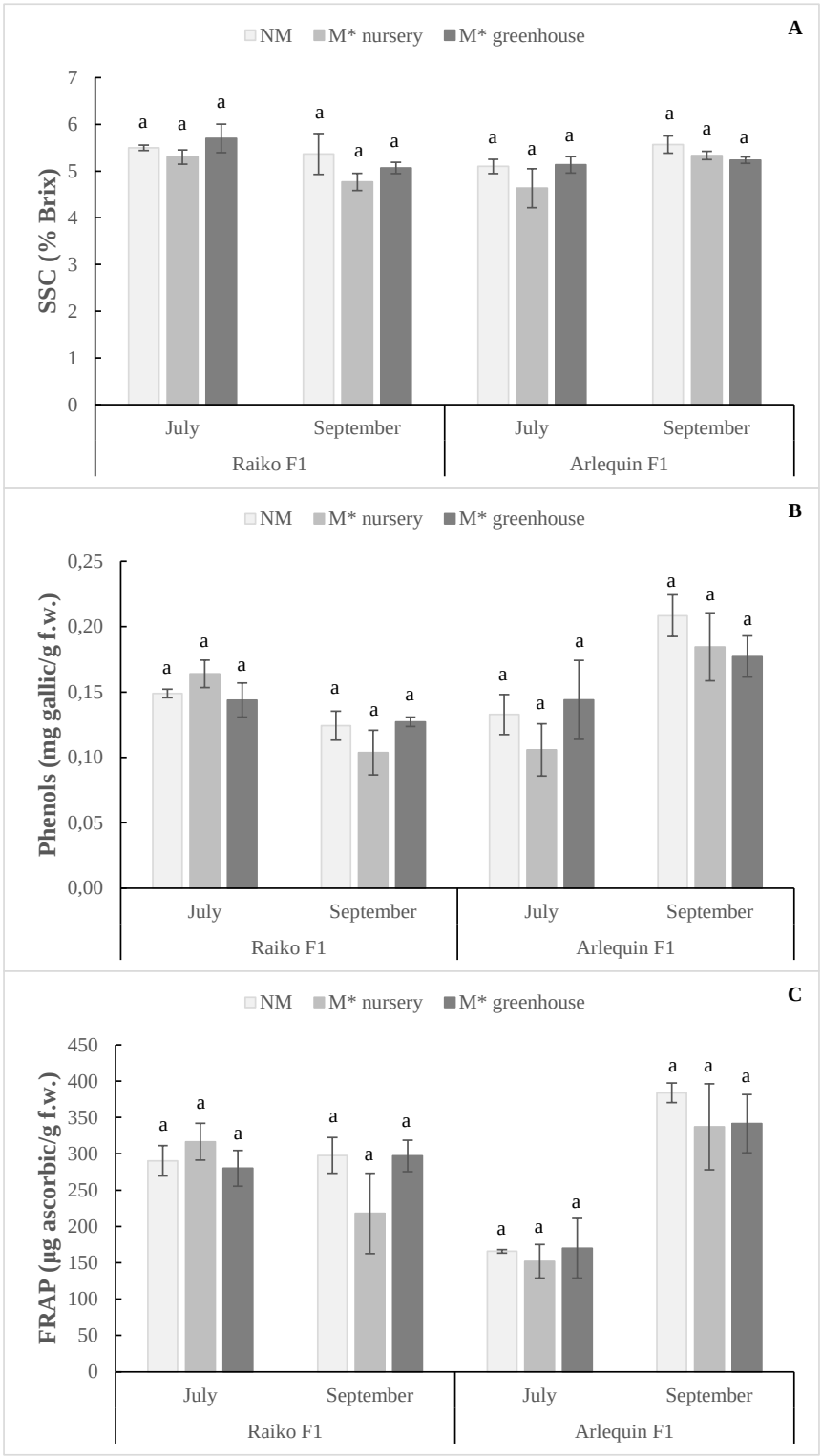
Cultivars	Yield (kg/plant)		Number of fruit/plant		Early yield (kg/plant)	
	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin
NM*(control)	1.65b	1.21a	19.2b	17.7a	0.36a	0.22a
M** at nursery	1.88b	1.07a	22.5b	15.8a	0.33a	0.22a
M** at greenhouse	2.71a	1.25a	31.2a	18.1a	0.37a	0.25a

*NM – non-mycorrhizal plants. **M – mycorrhizal plants. Values followed by the same letter do not significantly differ between the treatments. Statistical analysis was done separately for each hybrid

Table 8. Arbuscular mycorrhizal fungal (AMF) effect color development of organic pepper fruits

Mycorrhizal treatments	L*				Chroma				Hue			
	July		September		July		September		July		September	
Cultivars	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin	Raiko	Arlequin
NM* (control)	51.98a	55.19a	30.69a	42.73a	27.05a	40.08a	49.54a	57.60a	111.4a	111.6a	113.8a	112.3a
M** at nursery	49.30b	54.83a	27.65a	41.93a	28.07a	39.58a	48.25a	57.64a	111.2a	112.2a	113.0a	112.6a
M** at greenhouse	48.46b	56.58a	30.96a	42.64a	28.08a	38.28a	49.47a	56.32a	112.1a	111.9a	112.9a	112.9a

*NM – non-mycorrhizal plants. **M – mycorrhizal plants. Values followed by the same letter do not significantly differ between the treatments. Statistical analysis was done separately for each hybrid



NM – non-mycorrhizal plants. *M – mycorrhizal plants

Fig. 1. Soluble solid content (A), total phenolic content (B) and antioxidant activity (C) in NM and M pepper fruits during vegetation

DISCUSSION

High soil P is known to suppress the mycorrhizal symbiosis. It has been shown in several cases that an additional stress may allow the symbiosis to function efficiently at high soil P concentrations [Sylvia et al. 1993, Douds et al. 2012]. Commercial greenhouses often have high soil salinity and high soil P concentrations and therefore provide an environment where application of AMF inocula may be successful in high input agriculture. Therefore, a full-scale greenhouse experiment was employed to assay the AMF inoculation under realistic conditions of high soil P and salinity, and to focus on pepper fruit yield and other growth and nutritional variables. However, the use of a commercial greenhouse has the limitation of a lack of a non-saline control; we did not test whether our inoculum would have also been efficient under the same conditions in that particular high P soil, but without salinity.

In order to minimize the negative effects of high P conditions on the establishment of AMF symbiosis, pepper plants were pre-inoculated and subsequently transplanted. Such an inoculation strategy has been proposed as a potential way to overcome limitations for using the AMF biotechnology in high input agricultural systems, and to overcome competition with indigenous AMF community [Mummey et al. 2009]. It was anticipated that under this inoculation strategy, already established symbiosis would not be particularly affected by high soil P levels and could be beneficial for plant yield [Smith et al. 2011]. In this way, vegetable crop farmers who produce their own seedlings for later planting into the field, can more economically utilize inoculum of AM fungi by adding inoculum to the potting mix, in which seedlings are grown during the greenhouse phase of production. Furthermore, salinity is known to inhibit germination of AM fungal spores [Evelin et al. 2009], and pre-inoculation may help overcome this limitation in saline soils.

Alternatively, inoculation of AMF directly in the field at transplanting has been the most common application method in low-input systems. Following this approach, Ortas [2012] found higher fruit yield in pepper plants inoculated with AMF at transplantation. Further, Ortas et al. [2011] found that the inoculation of pepper plants with different AMF inocula resulted in higher shoot P concentration relative to the controls, but with no differences in shoot biomass.

The inoculum applied at nursery had poor colonization of young pepper plants of both cultivars at greenhouse soil transplantation. This could be attributed to late establishment (or early transplantation) or suppression of symbiosis due to high P levels of the potting substrate that contained 30% goat manure. The choice of the latter was based on the usual mode of operation in the particular greenhouse, and it is a good example of a need for management modifications required for successful AMF inoculum application. High concentrations of P in our soil most likely inhibited the root colonization of pepper plants at harvest. This probably suggests that root growth rate was faster than the spread of AMF and/or that high P levels also resulted in a slow growth of fungal infection units [Smith et al. 2011]. However, the percentage of fungal colonization is not always related to plant growth [Alberton et al. 2005], although in this study, pepper fruit P concentration was well correlated with root length colonization.

There was a substantial increase in pepper fruit yields for Raiko cv. when the inoculum was applied at transplantation. Although there is little information about beneficial role of mycorrhizal association in pepper species grown in soils subjected to organic manure, favorable results have been shown in some studies. Dai et al. [2011] showed that mycorrhizal inoculation increased the root (20%) and shoot (14%) dry mass, as well as N (12%) and P (12%) levels in the shoot. These responses increased significantly due to the amendment of soil with organic manure. Maximum root infection was seen at 100% of the recommended dose of organic manure application in soil. In addition, Boonlue et al. [2012] observed that mycorrhizal inoculation in organic systems increased growth parameters, including shoot height (41%), stem diameter (39%), fresh mass of shoots (85%) and roots (77%).

One important factor that may influence the extent of mycorrhizal colonization is temperature. Under moderate temperatures (20.7–25.4°C), mycorrhized pepper plants showed improvements in shoot dry mass and concentrations of P [Martin and Stutz 2004]. These effects were reduced at elevated temperatures (32.1–38°C). Warm soil conditions, there-

fore, surely alter AM fungal activity. The air temperature of the greenhouse, where the experiment was conducted, reached 42°C in summer, however, the effects of temperature on the rate and extent of colonization is complex and may vary with both the fungus and the plant.

The results of inclined biomass production and fruit yield are in accordance to numerous reports showing that AM inoculation enhanced the root and shoot dry weight, and fruit yield in pepper plants [Boonlue et al. 2012, Tanwar et al. 2013]. Several other studies have revealed no differences in pepper fruit yield between inoculated and non-inoculated plants [Russo and Perkins-Veazie 2010]. Some previous studies have indicated that plant responses to AMF inoculation could substantially vary among different cultivars of the same plant [Steinkellner et al. 2012]. For a given AMF inoculum and host, it is necessary to explore the optimum mode of application, which will maximize their plant bio-enhancing potential depending on cultivar, the local soil and environmental conditions.

Mycorrhizal inoculum application is expected to be successful when, among others, the mycorrhizal potential of indigenous AMF community is inadequate [Koide and Mosse 2004]. This was probably the case, since the applied inoculum was more effective than local mycorrhizal community, although levels of colonization at the end of the experiment were similar for both inoculated and non-inoculated plants. The lack of positive yield response in cv. Arlequin pepper plants could be attributed to very high soil P levels, but also to differentiation in symbiosis with different cultivars.

The effect of AM symbiosis on the quality attributes of fruits of *Capsicum* spp. has not been widely reported. Castillo et al. [2009] showed a slight increase in ascorbic acid concentration in fruits of colonized plants; however, no significant differences were found for total titratable acidity (% of citric acid) and total soluble solids (°Brix). Under drought conditions, Mena-Violante et al. [2006] found that the inoculation of pepper plants (cv. San Luis) promoted significant improvements in various fruit quality parameters, such as pigment concentration. Under water stress, chlorophyll content in fruit of mycorrhized plants was similar to that found in non-inoculated plants that were not subjected to drought. In addition, the levels of carotenes and xanthophylls in the fruit were 1−4 times higher in AM inoculated plants subjected to drought compared to that in non-inoculated plants not exposed to drought. This could be attributed to a mycorrhizal inoculation effect on the process of fruit maturation [Castillo et al. 2009] and changes in the concentration of photosynthetic pigments during symbiosis.

Arbuscular mycorrhizal fungi (AMF) may interact with host plant metabolism, inducing the accumulation of health-promoting phytochemicals and antioxidant molecules [Avio et al. 2017]. The TSP content was at the same level in fruits from inoculated plants compared to those from non-inoculated plants. In this study, TSP level in fruits of pepper during the growing season in infected and non-infected plants, depending on the variety, was variable (Fig. 1).

CONCLUSIONS

Beneficial microbial inoculants, such as AMF, make an attractive strategy to farmers in the context of organic and low input agriculture. However, results may vary with AM fungal species, cultivar, as well as agricultural practices. The intense use of manure as a fertilizer may lead to high soil P concentration and soil salinity, with each of these conditions being known to suppress the symbiosis. Results of this study are an indication that high soil P concentration combined with a plant stress, such as soil salinity, may enhance the effectiveness of mycorrhizal symbiosis.

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REFERENCES

Abdel Latef, A.A. (2013). Growth and some physiological activities of pepper (*Capsicum annuum* L.) in response to cadmium stress and mycorrhizal symbiosis. J. Agric. Sci. Technol., 15, 1437–1448.

Alberton, O., Kuyper, T.W., Gorissen, A. (2005). Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO_2. New Phytol., 167, 959–868.

Al-Karaki, G.N. (2017). Effects of mycorrhizal fungi inoculation on green pepper yield and mineral uptake under

irrigation with saline water. Adv. Plants Agric. Res., 6(5), 00231

Avio, L., Sbrana, C., Giovannetti, M., Frassinetti, S. (2017). Arbuscular mycorrhizal fungi affect total phenolics content and antioxidant activity in leaves of oak leaf lettuce varieties. Sci. Hortic., 224, 265–271.

Bagyaraj, D.J., Sharma, M.P., Maiti, D. (2015). Phosphorus nutrition of crops through arbuscular mycorrhizal fungi. Curr. Sci., 108, 1288–1293.

Baum, C., El-Tohamy, W., Gruda, N. (2015). Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. Sci. Hortic., 187, 131–141.

Beltrano, J., Ruscitti, M., Arango, M.C., Ronco, M. (2013). Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and P levels. J. Soil Sci. Plant Nutr., 13, 123–141.

Benzie, I.F.F., Strain, J.J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Meth. Enzymol., 299, 15–27.

Boonlue, S., Surapat, W., Pukahuta, C., Suwanarit, P., Suwanarit, A., Morinaga, T. (2012). Diversity and efficiency of arbuscular mycorrhizal fungi in soils from organic chili (*Capsicum frutescens*) farms. Mycoscience, 53, 10–16.

Bravo, A., Brands, M., Wewer, V., Dörman, P., Harrison, M.J. (2017). Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. New Phytol., 214, 1631–1645

Castillo, R.C., Sotomayor, S.L., Ortiz, O.C., Leonelli, C.G., Borie, B.F., Rubio, H.R. (2009). Effect of arbuscular mycorrhizal fungi on an ecological crop of chili peppers (*Capsicum annuum* L.). Chilean J. Agric. Res., 69, 79–87.

Dai, O., Singh, R.K., Nimasow, G. (2011). Effect of arbuscular mycorrhizal (AM) inoculation on growth of Chili plant in organic manure amended soil. Afr. J. Microb. Res., 5, 5004–5012.

Diaz Franco, A., Alvarado Carrillo, M., Ortiz Chairez, F., Grageda Cabrera, O. (2013). Plant nutrition and fruit quality of pepper associated with arbuscular mycorrhizal in greenhouse. Rev. Mex. Cienc Agríc., 4, 315–321.

Douds, D.D., Jr., Nagahashi, G., Reider, C., Hepperly, P.R. (2007). Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil. Biol. Agric. Hortic., 25, 67–78.

Douds, D.D., Lee, J., Rogers, L., Lohman, M.E., Pinzon, N., Ganser, S. (2012). Utilization of inoculum of AM fungi produced on-farm for the production of *Capsicum annuum*: A summary of seven years of field trials on a conventional vegetable farm. Biol. Agric. Hortic., 28, 129–145.

Duc, N.H., Mayer, Z., Pek, Z., Helyes, L., Posta, K. (2017). Combined inoculation of arbuscular mycorrhizal fungi, *Pseudomonas fluorescens* and *Trichoderma* spp. for enhancing defense enzymes and yield of three pepper cultivars. App Ecol. Environ. Res., 15, 1815–1829.

Evelin, H., Kapoor, R., Giri, B. (2009). Arbuscular mycorrihizal fungi in alleviation of salt stress: a review. Ann. Bot., 104, 1263–1280.

Hernádi, I., Sasvári, Z., Albrechtová, J., Vosátka, M., Posta, K. (2012). Arbuscular mycorrhizal inoculant increases yield of spice pepper and affects the indigenous fungal community in the field. HortScience, 47, 603–606.

Ipsilantis, I., Samourelis, C., Karpouzas, D.G. (2012). The impact of biological pesticides on arbuscular mycorrhizal fungi. Soil Biol. Biochem., 45, 147–155.

Jamiołkowska, A., Księżniak, A., Hetman, B., Kopacki, M., Skwaryło-Bednarz, B., Gałązka, A., Thanoon, A.H. (2017). Interactions of arbuscular mycorrhizal fungi with plants and soil microflora. Acta Sci. Pol. Hortorum Cultus, 16, 89–95.

Klironomos, J.N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology, 84, 2292–2301.

Koide, R.T., Mosse, R.T. (2004). A history of research on arbuscular mycorrhizal symbiosis. Mycorrhiza, 14, 145–163.

Maboko, M.M., Bertling, I., Du Plooy, C.P. (2013). Arbuscular mycorrhiza has limited effects on yield and quality of tomatoes grown under soilless cultivation. Acta Agric. Scand. Sec. B Soil Plant Sci., 63, 261–270.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.S., Swan, J.A. (1990). A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol., 115, 495–501.

Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., Niggli, U. (2000). Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. Biol. Fertil. Soils, 31, 150–156.

Martin, C.A., Stutz, J.C. (2004). Interactive effects of temperature and arbuscular mycorrhizal fungi on growth, P uptake and root respiration of *Capsicum annuum* L. Mycorrhiza, 14, 241–244.

Mena-Violante, H.G., Ocampo-Jimenez, O., Dendooven, L., Martinez-Soto, G., Gonzalez- Castaneda, J., Davies, F.T., Olalde-Portugal, V. (2006). Arbuscular mycorrhizal fungi enhanced fruit growth and quality of chile ancho (*Capsicum annuum* L. cv. San Luis) plants exposed to drought. Mycorrhiza, 16, 261–267.

Mummey, D.L., Antunes, P.M., Rillig, M.C. (2009). Arbuscular mycorrhiza fungi pre-inoculant identity determines community composition in roots. Soil Biol. Biochem., 41, 1173–1179.

Ortas, I., Sari, N., Akpinar, C., Yetisir, H. (2011). Screening mycorrhiza species for plant growth, P and Zn uptake in pepper seedling grown under greenhouse conditions. Sci. Hortic., 128, 92–98.

Ortas, I. (2012). The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. Field Crops Res., 125, 35–48.

Pellegrino, E., Bedini, S., Avio, L., Bonari, E., Giovannetti, M. (2011). Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil. Soil Biol. Biochem., 43, 367–376.

Pereira, J.A.P., Vieira, I.J.C., Freitas, M.S.M., Prins, C.L., Martins, M.A., Rodrigues, R. (2016). Effects of arbuscular mycorrhizal fungi on *Capsicum* spp. J. Agric. Sci., 154, 828–849.

Ruiz-Lozano, J.M., Aroca, R., Zamarreño, Á.M., Molina, S., Andreo-Jiménez, B., Porcel, R., García-Mina, J.M., Ruyter-Spira, C., López-Ráez, J.A. (2016). Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. Plant Cell Environ., 39, 441–452.

Russo, V.M., Perkins-Veazie, P. (2010). Yield and nutrient content of bell pepper pods from plants developed from seedlings inoculated, or not, with microorganisms. HortScience, 45, 352–358.

Scalbert, A., Monties, B., Janin, G. (1989). Tannins in wood: Comparison of different estimation methods. J. Agric. Food Chem., 37, 1324–1329.

Smith, S.E., Jakobsen, I., Grønlund, M., Smith, F.A. (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol., 156, 1050–1057.

Smith, F.A., Smith, S.E. (2011). What is the significance of the arbsucular mycorrhizal colonisation of many economically important crop plants? Plant Soil, 348, 63–79.

Steinkellner, S., Hage-Ahmed, K., García-Garrido, J.M., Illana, A., Ocampo, J.A., Vierheilig, H. (2012). A comparison of wild-type, old and modern tomato cultivars in the interaction with the arbuscular mycorrhizal fungus *Glomus mosseae* and the tomato pathogen *Fusarium oxysporum* sp. *lycopersici*. Mycorrhiza, 22, 189–194.

Sylvia, D.M., Hammond, L.C., Bennett, J.M., Haas, J.H., Linda, S. (1993). Field response of maize to a VAM fungus and water management. Agron. J., 85, 193–198.

Tanwar, A., Aggarwal, A., Kadian, N., Gupta, A. (2013). Arbuscular mycorrhizal inoculation and super phosphate application influence plant growth and yield of *Capsicum annuum*. J. Soil Sci. Plant Nutr., 13, 55–66.

Vincente-Sánchez, J., Nicolás, E., Pedrero, F., Alarcón, J.J., Maestre-Valero, J.F., Fernández, F. (2014). Arbuscular mycorrhizal symbiosis alleviates detrimental effects of saline reclaimed water in lettuce plants. Mycorrhiza, 24, 339–348.

Vosátka, M., Albrechtová, J., Patten, R. (2008). The international market development for mycorrhizal technology. In: Mycorrhiza, Varma, A. (ed.). Springer-Verlag, Berlin, 419–438.

Zhu, X.Q., Wang, C.Y., Chen, H., Tang, M. (2014). Effects of arbuscular mycorrhizal fungi on photosynthesis, carbon content and calorific value of black locust seedlings. Photosynthetica, 52, 247–252.