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Real-case application of mycorrhizal inoculums on *Capsicum annuum* L. var. *longum* cv. Szegedi and Kalocsai

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Summary: The aim of this study was to test the use of commercially available arbuscular mycorrhiza (AM) inoculant Symbivit, a mixture of six species of *Glomus* spp., in spice pepper field cultivation. The inoculants containing arbuscular mycorrhizal fungi (AMF) was able to establish a symbiosis in the rhizosphere of pepper plants and mycorrhizal inoculation increased fresh and dry weights of shoots of spice pepper cv. Szegedi and only fresh weight of Kalocsai type. There were no significant differences in the root weights due to treatment only in fresh weight of Kalocsai pepper type. Treated plants of both variants exhibited an increase in cumulative crop production compared with control non-treated plants and the growth response of pepper was higher for var. Szegedi than var. Kalocsai. Mycorrhizal inoculation had a great positive effect on external hyphal length of AMF also showing differences in that between Kalocsai and Szegedi variants. The root colonization showed seasonality by treated and non-treated plants. The lowest degree of colonization was observed in June in general and colonization percent increased during vegetative development and there was a slight decrease at harvesting. In conclusion, it can be stated that inoculation with Symbivit containing mycorrhizal fungi could be an integral part of spice pepper production.

Keywords: arbuscular mycorrhiza, hyphae, inoculation, pepper, yield

Introduction

Soil organisms, together with mycorrhizal fungi play a crucial role in the functioning of agricultural ecosystems. One type of mycorrhizal association, arbuscular mycorrhizal (AM) symbiosis, can contribute significantly to plant nutrition by promoting the uptake of phosphorus and nitrogen (*George, 2000*) resulting in improved plant growth and health. Colonization by AM fungi may also improve plant establishment, enhance plant tolerance to biotic and abiotic stress (*Schützendübel & Polle, 2002*) therefore growing interest in mycorrhizal inoculation in cost-efficient plant production in horticulture is clear. The interest in use of mycorrhiza in horticulture is mostly due not only to enhance uptake of P and water but decrease agrochemical inputs during production, and increase in vegetable nutritional quality via activation of antioxidant, vitamins or carotenoid pathways (*Baslam et al., 2011*).

Early evidence of the positive influence of the AM symbiosis on horticultural production was firstly provided by *Menge et al*. (1977). According to his work inoculation of horticultural plants with arbuscular mycorrhizal fungi (AMF) can (i) improve rooting and plant establishment; (ii) increase uptake of some ions and influence nutrient cycling; (iii) enhance plant tolerance to (biotic and abiotic) stress; (iv) improve the quality of soil structure; (v) promote earlier flowering and fruiting; and (vi) increase crop uniformity.

However the effects of AM on the growth and development of horticultural crop plants have been described in many research papers (*Azcón-Aguilar & Barea 1997; Lovato et al., 1995*) but research focused on typical vegetable from Hungary is missing.

Hungary is known for spice pepper (*Capsicum annuum* L. var. *longum*), which is protected by EU as a national cultivar. It is traditionally cultivated in the Szeged and Kalocsa regions of Hungary (*Somogyi et al., 2000)* and about 28.6 thousand tons are annually produced from 1,500 ha (average 2000-2009). A growing awareness of sustainable agriculture, high-quality food, and more information on how food is produced has caused a demand for reduced chemical inputs in pepper cultivation also.

The aim of the present study was to evaluate a possible sustainable, ecological way of producing mycorrhizal inoculums Symbivit (Symbio-m, CZ) on growth and yield of field grown spice pepper.

Material and methods

Experimental design

The experiment was carried out at the Experimental Station of Szent István University Gödöllő, Hungary (longitude, 19°21′39′, latitude, 47°35′37′). The climate of the region is continental with a mean annual temperature and precipitation of 10.6°C and 539 mm, respectively. Seedlings of pepper (*Capsicum annuum* L. var. *longum*), cv. Szegedi and cv. Kalocsai, were propagated at the beginning of April in a greenhouse using special horticulture substrate (Klasmann TS3: 80% white sphagnum peat and 20% frozen black sphagnum peat, slow-release 14:16:18 (w/w/w) NPK fertilizer, pH 6.00) for 7 weeks and were bedded out on 26 May in a sandy soil with medium phosphorus supply. The major soil properties in the 0–20 cm layer were: pH (KCl): 6.67; humus content: 1.56; salt: 0.05%, AL-P_2O_5: 84.0 mg·kg^{-1}; AL-K_2O: 113.0 mg·kg^{-1}; AL-Ca: 1347.0 mg·kg^{-1}, AL-Mg 142.0 mg·kg^{-1}, NH_4^+-N: 0.59 mg/100 g, NO_3^--N: 2.19 mg/100 g Cu: 3.63 mg·kg^{-1}, Fe 1016.0 mg·kg^{-1}, Mn 263.0 mg·kg^{-1}, Zn 6.49 mg·kg^{-1}. A variety of cereals have been grown alternatively on the site, however the last two years covered by grass/cover to restore soil fertility. A moldboard plough to 25 cm depth was used for soil tillage after each harvesting time and conventional seedbeds were prepared by chisel plowing followed by disking.

Before transplanting mycorrhizal fungi in a commercial product Symbivit® (mixture of *G. intraradices* BEG140, *G. mosseae* BEG95, *G. etunicatum* BEG92, *G. claroideum* BEG96, *G. microaggregatum* BEG56, *G. geosporum* BEG199, without bioadditives) produced by Symbiom Ltd. (Lanskroun, Czech Republic; www.symbiom.cz) was applied at 15 g of inoculum per pepper seedling into the planting hole and seedlings were planted immediately. In the experiment 50 plants of each pepper types inoculated with Symbivit (AM-treated) and 50 non-inoculated plants (Non-treated) were set up in three replicates using randomized block design. Five randomly chosen plants from each treatment were destructively harvested at the end of the experiment (on 3 September), shoots and roots, were dried at 60 °C for 72 h and separately weighed. Harvesting was by hand twice and cumulative weight of yield from 100 plants per treatment evaluated.

Assessment of mycorrhizal colonization and hyphal length of AMF

Samples for estimating root colonization were collected on 26 June, 17 July, 27 July, 14 August and 3 September. At each time three randomly chosen from three repetitive plots of the same treatment plants were dug out with a soil core of 25 × 25 × 25 cm. The roots and the soil were stored in separate plastic bags at 4°C until processing within 24 h. Approximately, half of the root systems, at least 500 mg of fine roots from each plant were transferred to separate tubes and were subjected to Trypan Blue staining (*Phillips & Hayman, 1970*). Internal fungal structures (hypae, arbuscules, vesicules) were examined under a stereomicroscope at × 100 magnification and the percentage of root length colonized calculated using the gridline intersect method (*Giovannetti & Mosse*, *1980*).

Determination of AM fungal hyphal length in the soil was based on the methods of *Baath & Söderstrom (1979).* A 1-g sample of dry rhizosphere soil from the last harvesting was dispersed in 100 mL deionized water in a blender for 1 min. Fungal hyphae were separated by wet-sieving and centrifugation. The separated fungal hyphae were placed in a Petri dish with 5 mL deionized water. Ten millilitres of agar solution (0.75%) containing trypan blue (0.05%) was added to each dish, and the mixture was then dried for 24 h at 70°C. The hyphal length was measured in the dried agar film by the intersection method *(Tennant, 1975)* under a binocular microscope (16 x magnification).

Data were analyzed by a one-way analysis of variance. Individual treatment differences were subjected to *t* test. Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, OK, USA) software.

Results and discussion

Mycorrhizal technology is being used more frequently in horticultural vegetable production (*Vosátka & Albrechtová*, 2008) and an international mycorrhizal industry is developing (*Vosátka et al.,* 2008). Under the threat of a depletion of world phosphate deposits, use of mycorrhizal inoculation in horticulture becomes even more desirable due to savings in chemical inputs and use of renewable materials in vegetable production.

Our results showed that the mycorrhizal AM-based commercial inoculant Symbivit was able to establish in roots and increased growth and yield of both spice pepper types. Mycorrhizal inoculation increased fresh (Figure 1.) and dry weights of shoots of spice pepper cv. Szegedi and only fresh weight of Kalocsai type (Figure 2., Table 1). There were no significant differences in the root weights due to treatment only in fresh weight of Kalocsai pepper type (Table 1.).

At each sampling date, except for that in July, the percent of mycorrhizal root colonization was greater for the AM-treated and non-treated plants (Figure 3A, 3B). However the relative high percentage of root colonization in the control non-inoculated treatment indicated the presence of indigenous populations of AMF in the soil. The root colonization showed seasonality by treated and non-treated plants. The lowest degree of colonization was observed in June in general and colonization percent increased during vegetative development and there was a slight decrease at harvesting.

Treated plants of both variants exhibited an increase in cumulative crop production compared with control non-treated plants. Inoculation with a commercial product containing a mixture of non-indigenous *Glomus* spp. could promote yield benefits of spice pepper cv. Szegedi by more

Table 1. Effects of commercial mycorrhizal inoculants on growth, external hyphal length of AMF and cumulative crop production during cultivation of spice pepper cv. Szegedi and cv.Kalocsai

Treatments	Fresh weight [g plant^{-1}]		Dry weight [g plant^{-1}]		External hyphal length	Cumulative crop production
	Root	Shoot	Root	Shoot	[m soil g^{-1}]	[g 100 plants^{-1}]
Szegedi						
AM-treated	1,66a	19,32b	1,30a	13,45b	8,32b	5251,27
Non-treated	1,77a	13,22a	0,97a	7,09a	5,65a	3189,56
Kalocsai						
AM-treated	3,38*b*	18,69*b*	2,18*a*	11,76*a*	6,75*b*	1126,11
Non-treated	2,16*a*	15,05*a*	1,48*a*	9,19*a*	4,81*a*	1032,95

Values followed by the same letter are not significantly different, P≤0.05, Tukey test. Values are means of five observations.

Figure 1. AM-treated and non-treated *Capsicum annuum* L. var. *longum* cv. Szegedi.

than 60 % and by 10 % of Kalocsai. The increase in pepper yield as a consequence of inoculation was observed by other authors (*Gaur et al.,* 1998; *Douds & Reider*, 2003; *Kaya et al.,* 2009; *Russo & Perkins-Veazie*, 2010) but not in spice pepper.

Mycorrhizal inoculation had a great positive effect on hyphal length also (Table 1) and there were differences between Kalocsai and Szegedi variants. According to the increased yield benefit, the same tendency was observed: spice pepper cv. Szeged showed higher values in hyphal length than Kalocsai pepper type. Extraradical hyphal length in the soil is directly related to root colonization, though colonization rates can be a poor predictor of extraradical hyphal length (*Bingham & Biondini*, 2009). As the plant allocates photosynthates to the fungus, it promotes hyphal growth inside and outside of the root, and the extension of the extraradical hyphae results in greater nutrient transfer from soil to the plant (*Lebron et al.,* 2012). In experimental chambers, the external hyphae of AM can deliver up to 80% of plant P, 25% of plant N, 10% of plant K, 25% of plant Zn and 60% of plant Cu (*Marschner & Dell, 1994)*. Nutrient uptake by mycorrhizal inoculants was not measured in our experiments, but the increased growth of treated spice pepper together with increased root colonization and hyphal length really show their role in pepper cultivation.

Besides the activity of external hyphae of AMF, arbuscular mycorrhizal fungi directly or indirectly affect the soil ecosystem through multiple interactions with other soil organisms. Although they are not saprotrophs, AMF can enhance the rate of decomposition of organic material (*Hodge et al.,* 2001), indirectly influencing decomposition

Figure 2. AM-treated and non-treated *Capsicum annuum* L. var. *longum* cv. Kalocsai.

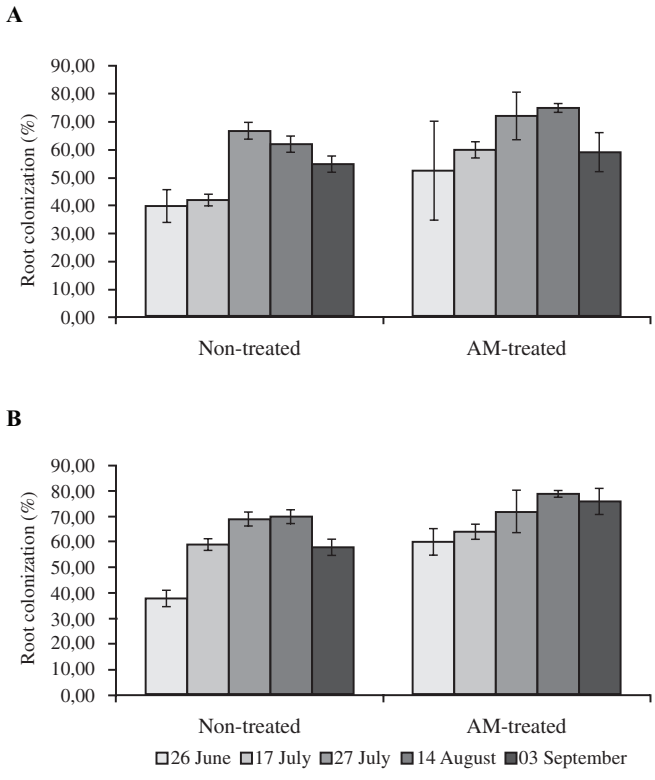

Figure 3. Colonization of pepper roots by AM fungi as affected by sampling times and pepper variants. A: *Capsicum annuum* L. var. *longum* cv. Kalocsai, B: *Capsicum annuum* L. var. *longum* cv. Szegedi

through interactions with other microorganisms in the soil. Some reports show that PGPR (plant growth promotion rhizobacteria), involved in nutrient cycling, have a strong stimulatory impact on the growth of AM fungi (*Andrade et al., 1997*) and vice versa, AMF could increase the concentration of some microorganisms (*Meyer & Lindermann 1986*). Moreover many rhizobacteria, for example phosphate solubilizing bacteria (PSB) and fungi are able to solubilize sparingly soluble phosphates, usually by releasing chelating organic acids (*Vessey, 2003*) and/or producing phosphatases for mobilization of organic phosphorous which influence the nutrient uptake of plants.

Plant genotype selection for reduced functioning of AM symbiosis under agricultural intensification was postulated long time ago (*Johnson, 1993*), but our results suggest that plant breeding by pepper does not select against the response to AMF, as has been suggested before for other cultivated species, e.g. wheat (*Sawers et al., 2004*). However differences in growth response caused by mycorrhizal inoculants between different pepper types found, the percentage of mycorrhizal root colonization assessed by Trypan Blue staining was not significantly different between samples collected from AM-treated plants.

Though introduction of non-native AMF is regarded as benign by some (*Azcón-Aguilar & Barea, 1997*), more concern has been given to introduction of exogeneous organisms since biological invasions can negatively affect local communities (*Koch et al., 2011*). On the basis of measuring root colonization only the occurrence of AMF could be detected but the increased yield benefit together with increased hyphal length due to mycorrhizal inoculation show that AMF from inoculants were able to establish and affect vegetable plants.

In conclusion, it can be stated that inoculation with Symbivit containing mycorrhizal fungi could be an integral part of spice pepper production.

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