https://doi.org/10.17221/4/2019-HORTSCI

Effect of aboveground plant conditioner treatment on arbuscular mycorrhizal colonization of tomato and pepper

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Citation: Lukács A.F., Kovács G.M. (2019): Effect of aboveground plant conditioner treatment on arbuscular mycorrhizal colonization of tomato and pepper. Hort. Sci. (Prague), 46: 208–214.

Abstract: We aimed to test the hypothesis that treatment with an aboveground plant conditioner has an effect on important vegetable crops inoculated with arbuscular mycorrhizal fungi (AMF) and on their colonization by AMF. Potting experiments were set with pepper and tomato plants inoculated with commercial AMF inoculum and plants were treated with an aboveground plant conditioner. After harvesting, the dry weight of shoots and roots were measured, and the AMF colonization of the roots was quantified. We found a significant effect of the treatment on fungal colonization: the AMF colonization, the hyphal colonization rate and the frequency of the arbuscules in the roots of both vegetables were lower when aboveground plant conditioner was applied. Although the two species differed, no significant effect of the treatment on the growth of the plants was detected. Based on our findings we assumed that the lower AMF colonization more greatly influenced the growth of the pepper cultivar studied. We demonstrated that treatment with a commercial aboveground plant conditioner had an antagonistic effect on AMF colonization, which, in addition to many other effects, might influence the growth vegetable crops. The interaction of different practices applied simultaneously should be tested to effectively help the development suitable agriculture systems.

Keywords: biopharming; horticulture; inoculation; symbiosis; vegetables

Today, 40% of the land area of the world is used for agricultural practice. In 1961, agricultural output was $746 billion, and production took place on 45 billion hectares of land to feed 3 billion people. Over the subsequent half-century, the world population grew by 1.69% per year, which meant that by 2011, 7 billion people needed to be fed. Along with this population expansion, the agricultural output increased more than 3-fold, to $2.4 trillion in 2011 (Alston, Pardey 2014).

Tomatoes and peppers are among the most important vegetable crops in the European Union. According to the Food and Agriculture Organization of the United Nations (FAO), the production of tomatoes and peppers in the European Union in 2013 was more than 15 million and 2 million tons, respectively (FAO 2013, http://faostat3.fao.org). Tomato and pepper are mostly cultivated in intensive agriculture systems, which use several fertilizers and apply several methods of plant protection. However, it is important for sustainable agriculture to study and develop methods with potential use in organic farming systems.

There are several commercial products licensed for use in agriculture systems, such as plant protection and nutrient products made from seaweed (the main groups are *Ascophyllum nodosum*, *Ecklonia maxima*, *Durvillea potatorum*, *Fucus serratus*, *Laminaria* sp. and *Macrocystis pyrifera*), believed to improve the efficiency of plant protection systems (Sharma et al. 2015).

Supported by the Hungarian Research Institute of Organic Agriculture (ÖMKI).

https://doi.org/10.17221/4/2019-HORTSCI

Several positive effects of these products have been demonstrated: application of a commercial product containing *Ascophyllum nodosum* extract increased fruit weight and maturation of kiwi (Chouliaras et al. 1995), improved fruit yield and fruit setting of grapevine (Colapietra, Alexander 2005; Khan et al. 2012), improved fruit yield of strawberry, blueberry, apple and olive (Basak 2008; Chouliaras et al. 2009; Spinelli et al. 2010; Loyola, Munoz 2011), improved iron deficiency tolerance of strawberry (Spinelli et al. 2010), and reduced the disease symptoms of potato caused by *Verticillium* (Uppal et al. 2008).

One European seaweed product is Alginure® (Tilco Biochemie GmbH), which is prepared from brown algae (*Ascophyllum nodosum* and *Laminaria* sp.) extract, mono-, oligo- and polysaccharides, plant amino acids, lipids, proteins, P_2O_5, K_2O and N. Furthermore, it has the ability to enhance the plant immune system and plant development (Boček et al. 2013). Based on previous studies, seaweed extract-based plant conditioners have a positive effect on plant defence against pathogens (Boček et al. 2013). The general defence mechanism might be improved against the colonization of the plant by pathogens as well as by mutualistic symbionts. However, to date, this has not been tested.

The majority of nutritionally relevant crops form mutualistic symbiosis via their root with obligate biotrophic fungi of the subphylum Glomeromycotina, called arbuscular mycorrhiza (AM) (Brundrett 2004). AM fungi (AMF) support water and nutrient uptake of plants and may help the plants to achieve resistance to abiotic and biotic stress. In exchange plants share assimilates with the fungi (Smith, Read 2010).

AM symbiosis offers multiple benefits to agricultural production systems: AMF inoculation of plants increases P uptake, growth and yield of crops (Gianinazzi et al. 2010), suppresses the competitive efficiency of aggressive weeds (Rinaudo et al. 2010), influences soil structure (Oehl et al. 2004), or induces resistance against pathogens (Klironomos 2002; Heere et al. 2007; Vos et al. 2012, 2013). AM inoculation has a positive effect on several important parameters of vegetable crops, and the application of AM inocula has been recommended as a suitable agricultural practice (Rouphael et al. 2015).

We studied whether a plant conditioner applied aboveground affected the AMF colonization of the roots of pepper and tomato. To test this hypothesis, we inoculated these vegetable crops with a commercially available AMF product and checked if the application of a commercial plant conditioner influenced their biomass production and AM colonization in their roots.

MATERIAL AND METHODS

Tomato (*Solanum lycopersicum* L., "Kecskeméti 549") and pepper (*Capsicum annuum* L., "Rapires F1") (both from "Zöldségtermesztési Kutató Intézet" (ZKI), Kecskemét, Hungary) seeds were surface sterilized by washing in 70% EtOH for 5 min and in 30% H_2O_2 for 1 min, then washed three times in sterilized tap water. After sterilization, the seeds were placed on wet filter paper in Petri-dishes for 10 days. The seedlings were planted into 450-ml pots (one plant per pot) filled with a mixture of sand and zeolite (2 : 1). The seedlings were inoculated with 20 ml of a commercial AMF inoculum (Symbivit®, Symbiom, Agrobio Hungary Ltd.) and watered with 80 ml of sterilized tap water.

Ten pepper and ten tomato plants were treated with Alginure® plant conditioner once every 2 weeks as recommended by the producer. The same number of each plant species served as controls without plant conditioner treatment. Plants were grown for 21 weeks at room temperature under natural light conditions and watered when needed. The pots were rearranged three times a week.

After 21 weeks, the plants, in their flowering period, were harvested. To check AM colonization, roots of approximately 100 cm total length were removed from each plant. Correction of total root biomass with the root removed was not needed; based on our pilot study, the removal of such an amount of roots had no significant effect on the total root mass (*data not published*). Shoot and root were separated and dried for 2 weeks at 65°C, and the dry biomass of shoot and root were measured.

The separated root segments were stained using the ink-vinegar method (Vierheilig et al. 1998) previously optimized to tomato and pepper. The roots of tomato were kept at 90°C for 10 min in 10% KOH and washed three times with distilled water. The roots of pepper were kept at 90°C for 70 min in 10% KOH and washed three times with distilled water. The washed roots of both species were treated by 5% acetic acid. Roots were stained in 5% ink (black ink, Schaeffer) acetic-acid solu-

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tion at 90°C for 8 minutes. The stained roots were washed in 5% acetic acid and mounted in lactic acid (90%). The AMF colonization was measured by the "magnified intersection method" (McGonigle et al. 1990). For quantification of the colonization, 30 pie-ces of 1-cm-long root segments of each plant were mounted on three slides (10 root segments on each) as recommended in McGonigle et al. (1990), and 100–150 intersections per slide were checked per plant.

The presence of arbuscules, vesicles and non-septated AMF hyphae were counted in each intersection when these structures were detected. The intensity of AMF colonization (ratio/percent of arbuscular colonization (A%), ratio/percent of vesicular colonization (V%), ratio/percent of hyphal colonization (H%) was quantified as the number of intersections (×100 for %) with arbuscules. Vesicles or hyphae were divided by the total number of intersections studied.

Because our response variables (colonization of roots (A%, V%, H%), dry biomass of shoots, roots, and total biomass) were not independent, we analysed them by multivariate analysis of variance (MANOVA). We entered plant species (tomato and pepper), treatment (conditioner and control) and their interaction as fixed predictors to the model. We ran the model in the Statistica program Version 10 (StatSoft Inc).

RESULTS

All plants survived the 21-week-long treatment and all plants appeared healthy. The ink-vinegar staining method was successful; all of the stained roots were suitable for quantification of the colonization. The inoculation was also successful; AM structures were detected in roots of all pepper and tomato plants.

Multivariate statistical analysis showed that plant species (Wilks $\lambda_{5,32}$ = 0.21*;* P < 0.001) and treatment (Wilks $\lambda_{5,32}$ = 0.46; P < 0.001) had significant effects without significant interaction (Wilks $\lambda_{5,32}$ = 0.92; P = 0.719). The subsequent univariate analyses showed that the treatment had a significant effect on the percent of arbuscular colonization (A%) ($F_{1,36}$ = 20.38; P < 0.001) and hyphal colonization (H%) ($F_{1,36}$ = 21.65; P < 0.001) but not on vesicular colonization ($F_{1,36}$ = 2.87; P = 0.099). Plant species had a significant effect on those variables (A%: $F_{1,36}$ = 15.89; P < 0.001; H%: $F_{1,36}$ = 16.01; P < 0.001; V% $F_{1,36}$ = 49.96; P < 0.001). On dry biomass (DW) parameters only, the plant species had significant effects (total DW: $F_{1,36}$ = 10.87; P = 0.002; shoot DW: $F_{1,36}$ = 43.71, P < 0.001; root DW: $F_{1,36}$ = 7.60; P = 0.009). The treatment had no significant effect on those values (total DW: $F_{1,36}$ = 3.08; P = 0.088; shoot DW: $F_{1,36}$ = 1.93, P = 0.173; root DW: $F_{1,36}$ = 2.59; P = 0.116)

Tomato

There was significant effect of the plant conditioner treatment on the AMF colonization of tomato roots. The AMF colonization was lower in the roots of tomato plants treated with the conditioner compared with control plants, both arbuscular and hyphal colonization was higher in roots of untreated plants than in roots of plant conditioner treated tomato plants (Table 1, Fig. 1).

The plant conditioner treatment had no significant effect on shoot, root and total biomass of tomato plants (Table 1, Fig. 2).

Pepper

Treatment with the plant conditioner had a significant effect on the AMF colonization of pepper. More AMF structures were present in the root of

Table 1. Intensity of colonization by different arbuscular mycorrhizal structures and dry weight of pepper and tomato. Values present the mean and standard deviation calculated from ten replicates of each treatment of each plant; for significant effects see the results of the MANOVA in the text

Plant	Treatment	Intesity of colonization (%)			Dry weight (g)		
		arbuscules	vesicles	hyphae	total	shoot	root
Pepper	conditioner	73.20 ± 11.59	64.09 ± 14.56	84.85 ± 5.48	4.031 ± 0.525	2.736 ± 0.396	1.295 ± 0.237
	control	86.63 ± 11.01	68.45 ± 17.72	91.64 ± 6.21	4.575 ± 0.299	3.069 ± 0.256	1.506 ± 0.237
Tomato	conditioner	59.37 ± 9.25	31.22 ± 10.27	76.06 ± 6.09	4.836 ± 0.555	3.688 ± 0.420	1.148 ± 0.221
	control	74.90 ± 8.37	41.26 ± 9.50	86.02 ± 4.90	4.886 ± 0.687	3.685 ± 0.404	1.201 ± 0.329

https://doi.org/10.17221/4/2019-HORTSCI

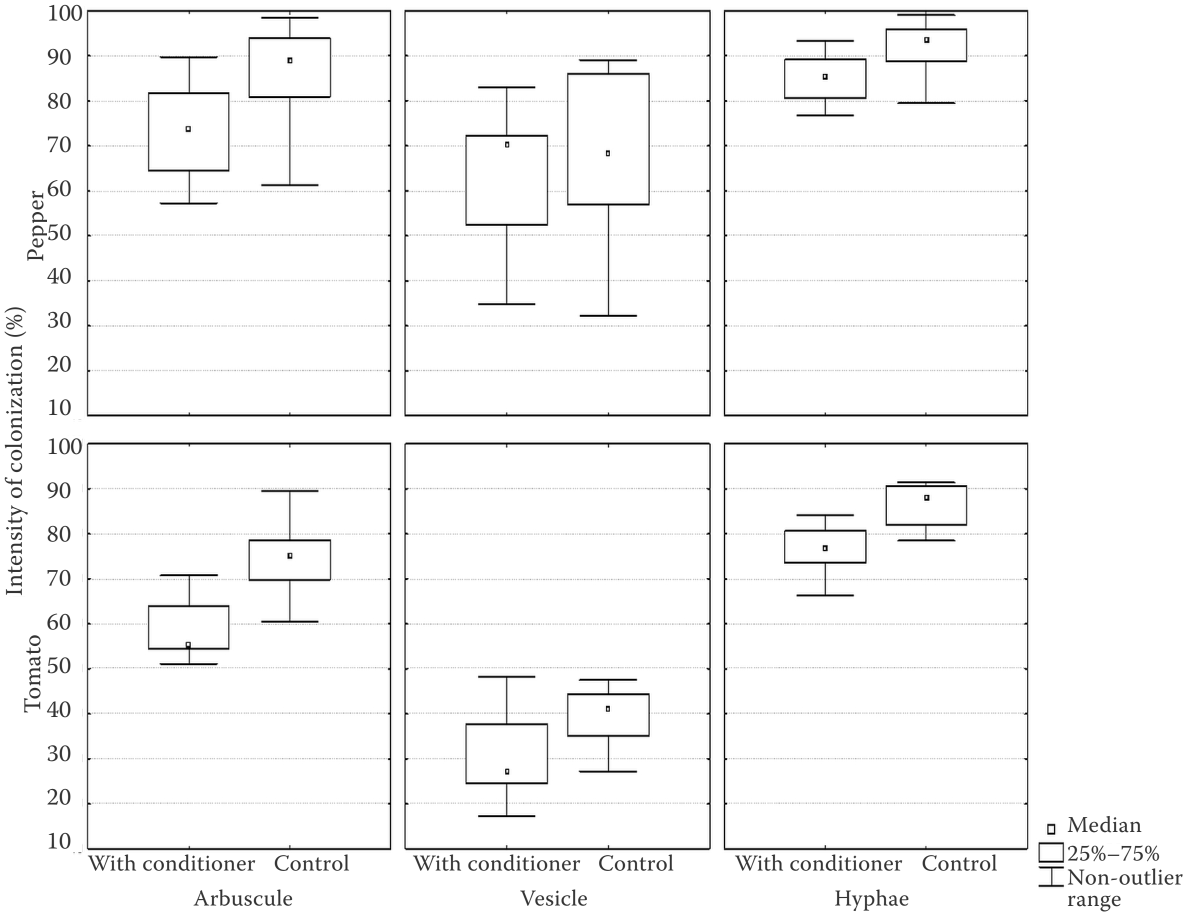

Fig. 1. Intensity of colonization by different arbuscular mycorrhizal structures (arbuscules, vesicles, hyphae) in the roots of AMF inoculated pepper and tomato plants with and without treatment with an aboveground conditioner. Intensity is calculated as the percent of the frequency of the certain structures quantified by magnified intersection method. For significant effects see the results of the MANOVA in the text

control plants than in treated ones. The colonization rate of arbuscules and hyphae was higher in the roots of the control plants than in treated pepper roots (Table 1, Fig. 1).

The plant conditioner treatment had no significant effect on shoot, root and total biomass of pepper plants (Table 1, Fig. 2).

DISCUSSION

Agricultural and horticultural practice commonly use nutrients, soil amendments and biostimulants to increase yield, productivity and plant defence mechanisms against different pathogens (du Jardin 2015). However, the simultaneous application of more than one of these products can have synergistic or antagonistic effects.

During this work, we studied the effects of a seaweed extract based commercial plant conditioner product applied aboveground on the AMF colonization and biomass productivity in tomato and pepper plants inoculated with a commercially available AM product.

Arbuscules and hyphae are indicators generally used to quantify the colonization efficiency of AMF because these two structures could be connected with the interaction of the partners in this mutualistic symbiosis (Parniske 2008).

Our results indicate that the application of the aboveground plant conditioner significantly decreased the number of those AM structures, i.e., the number of arbuscular and hyphal colonization rates in both tomato and pepper plants. No significant interaction between the plant species and the treatment was revealed in the statistical analysis, which

https://doi.org/10.17221/4/2019-HORTSCI

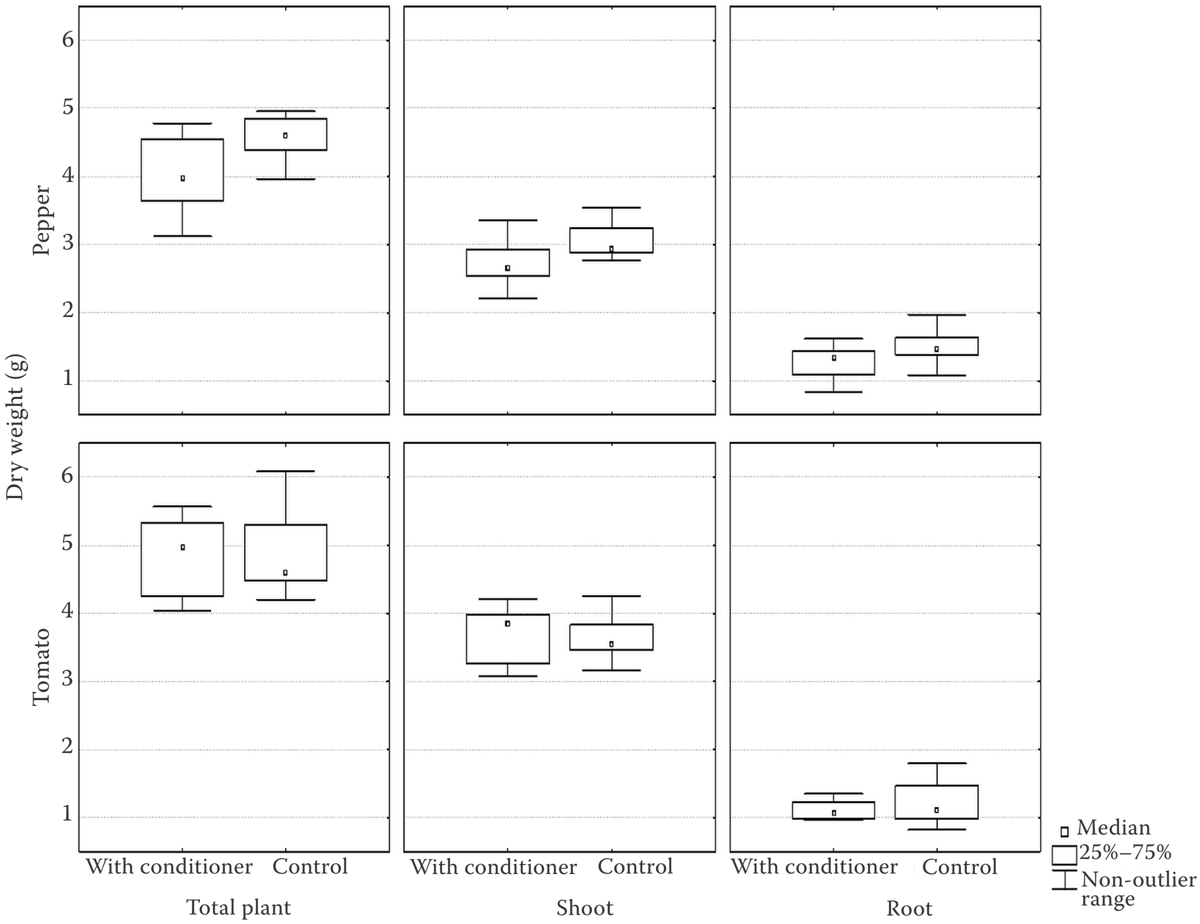

Fig. 2. Dry weight of total plant, shoot and root of AMF inoculated pepper and tomato plants with and without treatment with an aboveground conditioner. The dry weight was measured after 21 weeks of the experiment. For significant effects see the results of the MANOVA in the text

showed that the negative effect on those rates appeared independently from the plant species.

The plant conditioner treatment had a less obvious but different effect on the growth of pepper and tomato. As a trend, the biomass of nontreated pepper plants was higher than the ones where the plant conditioner was applied. This effect might be related to the different dependences of the two species on mycorrhizal symbioses. The effect of AM inoculation on pepper and tomato was studied previously and differences were found between the two crops (Ortas et al. 2003; Regvar et al. 2003). In our study, the conditioner reduced the AM colonization of both species, and this might have different effects on vegetable crops. Based on our findings, we assume that the tomato cultivar studied was less AM dependent than the pepper cultivar.

This lack of synergistic action can be attributed to several factors or acting mechanisms.

Plant immune reactions can be triggered by pathogens via recognition of pathogen-associated molecular patterns (PAMPs) by their receptors (pattern recognition receptors, PRRs) on the cell surface or by intracellular detection of effectors produced by the pathogens. Responses can be various, e.g. a rapid influx of calcium ions from external stores, a burst of active oxygen species, activation of mitogen-activated proteins, cell wall extension by deposition of callose to the cell wall or localized cell death (Dodds, Rathjen 2010).

The main cell wall polysaccharides of brown algae are alginates, fucoidan and laminarin (Rioux et al. 2007). Elicitor activity of laminarin has been shown in grapevine cell suspension. After laminarin application, oxidative burst, calcium ion influx, rapid activation of MAPKs and expression of defence-related genes have been described (Aziz et al. 2003).

https://doi.org/10.17221/4/2019-HORTSCI

The process of AMF colonization is regulated by AMF and the plant partner (Genre et al. 2008). The elicitor activity of some polysaccharides of brown algae can stimulate the immune system of plants causing cell wall thickening and molecular changes in root cells. These changes may generate a loss of AMF colonization efficiency of treated plants (Vera et al. 2011).

According to Cheng et al. (2013), P-fertilizers have a major impact on AMF communities on a long-time scale, and there was lower AMF colonization in the P-fertilized pots than in the P-unfertilized pots. Mäder et al. (2000) found that high-input agriculture management had a negative effect on AMF colonization activity. Qin et al. (2015) investigated the impact of organic or inorganic fertilizers on AMF growth in a long-term field experiment. They also found that different fertilizers altered the AMF communities, but they had no significant effect on the root colonization rate. The tested plant conditioner in this study contained high level of P (P_2O_5 – 20 ± 1.1 m/m%) which could be a cause of the loss of efficiency of AMF colonization in pepper and tomato plants.

CONCLUSION

Factors affecting AMF activity and communities are complex and not well understood. In addition, they might have huge practical importance in horticulture. Here, we reported how the simultaneous application of two agricultural products, namely inoculation with AMF and the application of an aboveground plant conditioner, influenced root colonization of pepper and tomato by plant growth-promoting microbes. The results highlight the importance of testing if there is any synergistic or antagonistic effect of the simultaneous application of different products.

Acknowledgement

The Authors thank Adrienn Micsinai's valuable comments on the manuscript, Dániel G. Knapp's help and useful suggestions and Gábor Herczeg for his help in statistical analyses.

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Received for publication January 7, 2019
Accepted after corrections July 13, 2019