

Mycorrhizal inoculation as an alternative in the ecological production of tomato (*Lycopersicon esculentum* Mill.)

Agnieszka Jamiołkowska¹, Ali Hamood Thanoon², Barbara Skwaryło-Bednarz^{1*}, Elżbieta Patkowska¹,
and Elżbieta Mielniczuk¹

¹Department of Plant Protection, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-069 Lublin, Poland

²Department of Plant Protection, College of Agriculture and Forestry, University of Mosul, 41002 Mosul, Iraq

Received October 24, 2019; accepted February 14, 2020

Abstract. The aim of study was to investigate the effect of two mycorrhizal fungus species *Claroideoglossum etunicatum* and *Rhizophagus intraradices* on the uptake of macronutrients and on the growth and yield of tomato hybrid plants cultivated in an ecological system. The experiment was carried out at an ecological farm in Grądy in the Lublin province of Poland, for three years (2015-2017). The experimental treatments included plants inoculated with *Claroideoglossum etunicatum*, *Rhizophagus intraradices* and plants without mycorrhizal inoculation used as a control. The mycorrhization of tomato roots with *Claroideoglossum etunicatum* and *Rhizophagus intraradices* resulted in an improved uptake of Ca and K through the plants. The length of the tomato roots, especially for the cultivars treated with *Claroideoglossum etunicatum*, were statistically longer than those of the control. Tomato roots inoculation with both of the studied mycorrhizal fungus strains significantly influenced the number of tomato leaves and improved the health status of the plant. The yield of tomato was not significantly affected by mycorrhization but *Claroideoglossum etunicatum*, to a greater extent than *Rhizophagus intraradices* reduced the yield of diseased fruit compared to the control. Among the studied mycorrhizal fungus species, better results were obtained with the application of *Claroideoglossum etunicatum* as compared with *Rhizophagus intraradices* for all examined features. Mycorrhizal inoculation contributed to the better growth of the plants, it improved their health and may be beneficially applied in the ecological production of tomatoes.

Keywords: nutrition, tomato, ecological production, mycorrhizal inoculation

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable plants in the world (Nicola *et al.*, 2009; Panthee and Chen, 2010; FAOSTAT, 2014). It is widely cultivated in tropical, subtropical and temperate climates and thus ranks third in terms of world vegetable production (FAOSTAT, 2014). Diseases are a major limiting factor for tomato production. The most important diseases are those caused by pathogens that include fungi, bacteria and viruses. These diseases are a major limiting factor and can be severe, reducing tomato yield and quality.

Due to the drawbacks of the chemical plant protection method, consumer pressure and the introduction of international food safety standards (IFSS), a need has arisen for farmers to change their production and marketing practices. As required by the IFSS, agricultural producers may consider it necessary to use alternative pest management methods, especially in ecological production. This production system takes into account biological methods, understood not only as direct protection, but also as the use of the natural processes of self-regulation. One of these methods is biological control, which provides environmentally safe ways to reduce the adverse effects of pathogens on crops. Potential biological control agents include arbuscular mycorrhizal fungi (AM Fungi) (Al-Askar and Rashad, 2010). AM Fungi are a promising source for improving sustainable food production and increasing human

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*Corresponding author e-mail: barbara.skwarylo@up.lublin.pl

nutritional needs (Hart *et al.*, 2015). AM Fungi used in horticulture are an effective means of improving plant growth (Oseni *et al.*, 2010). Mycorrhiza is a natural phenomenon offering many benefits for farmers and, therefore, may be useful in sustainable plant development (Conversa *et al.*, 2007; Candido *et al.*, 2015). AM Fungi are a major component of most plant rhizospheres and they play an important role in reducing the occurrence of plant diseases.

Different studies indicate that AM Fungi increase the nutrient uptake of plants. This improves the resistance of plants to pathogens through compensation for the symptoms of infection, and the morphological and biochemical changes in plants, as well as the increase in the number of antagonistic microorganisms in the rhizosphere (Kapoor, 2008; Song *et al.*, 2015). Mycorrhizal fungi stimulate plant defence reactions at the initial stages of symbiosis (Paszkowski, 2006). The modulation of plant defence during the establishment of mycorrhiza causes a pre-conditioning of tissues by effectively activating the defence mechanisms of the plants, which is known as the priming phenomenon (Pozo and Azcón-Aguilar, 2007). It plays a major role in mycorrhiza-induced resistance (Jung *et al.*, 2012). Song *et al.* (2015) found that plant mycorrhization increases tomato plant resistance to early infection by *Phytophthora infestans* by activating the jasmonic acid (JA) signaling pathway.

Numerous studies have confirmed that AM Fungi enhance plant resistance against various pathogens (Harrier and Watson, 2004; Pozo *et al.*, 2005; Bi *et al.*, 2007). Mycorrhizal colonization improved tomato resistance to wilt diseases caused by *Fusarium oxysporum* f. sp. *lycopersici* and increased tomato resistance to the foliar disease of early blight caused by *Alternaria solani* (Akköprü and Demir, 2005; Fritz *et al.*, 2006). The appropriate management of AM Fungi has the potential to provide sustainability in agricultural systems for crop disease management (Zeng, 2006; Liu *et al.*, 2007). However, the mechanism of disease resistance induced by AM Fungi remains elusive. It is well known that transcriptional reprogramming occurs in the host-plant upon mycorrhizal colonization (Jung *et al.*, 2012; Lopez-Ráez *et al.*, 2010). Mycorrhizal fungi control not only soil-borne pathogens but evidence has been presented which shows the suppression of leaf attacking pathogens. Fritz *et al.* (2006) found that the protective effect of mycorrhiza against the development of tomato leaf spots caused by *A. solani* has an effect equivalent to induced systemic resistance. Common mycorrhizal networks between tomato plants provide the protection of neighbours against early blight (Song *et al.*, 2015). In recent times, the system of the classification of fungi forming or considered to form arbuscular mycorrhiza placed them in phylum Glomeromycota and class *Glomeromycetes* with four orders (*Archaeosporales*, *Diversisporales*, *Glomerales*, and *Paraglomerales*), ten families and thirteen genera (Walker *et al.*, 2007; Palenzuela *et al.*, 2008; Oehl *et al.*, 2011; Błaszczowski, 2012).

Considering the above-mentioned aspects, it is believed that there is a need to study and select the species of mycorrhizal fungi which – when inoculated into the rhizosphere of tomato – will contribute to better plant nutrition, their improved growth and health status. Hence, the objectives of this study were to: I – determine the influence of AM Fungi on root colonization, II – investigate the variability of leaf nutrient content, growth and yield of tomato plants under the influence of the studied species of mycorrhizal fungi, III – determine which mycorrhizal fungus (MF) species will be better to use in the ecological production of tomato.

MATERIALS AND METHODS

The research work was carried out at an ecological farm located in Grądy in the Lublin province, Poland (51°05' N latitude, 22°12' E longitude) in the years 2015-2017. The object of the studies included three cultivars of tomato hybrids (*Lycopersicon esculentum* Mill.) important for commercial production: 'Antalya F1' (Yuksel Tohomculuk Company in Turkey), 'Esmira F1' (Rijk Zwaan Company in Holland), 'Pelikan F1' (Seed and Nursery Company in Ożarów Mazowiecki in Poland) and two species of mycorrhizal fungi (MF): *Claroideoglomus etunicatum* (CE) (syn. *Glomus etunicatum* W.N. Becker & Gerd) and *Rhizophagus intraradices* (RI) (syn. *Glomus intraradices* N.C. Schenck & G.S. Sm.). MF spores were provided from the collection belonging to the Department of Agricultural Microbiology, the Institute of Soil Science and Plant Cultivation – National Research Institute in Puławy, Poland. The experiment was established in a plastic tunnel with the dimension of 6×30×2.80 m. The experiment was carried out on albic luvisols (cutanic) soil (Kabała *et al.*, 2019). In each year, organic nitrogen fertilizer (Bioilsa 12.5 – NaturalCrop Poland) was used before planting plants in a permanent place using a dose of 500 kg ha⁻¹. Tomatoes were grown in soil with the following parameters: pH in H₂O 5.83-6.66 (KQ/PB-47); levels of available minerals: P-39.0-63.0 mg kg⁻¹ f.w. of soil (KQ/PB-51); K-314.0-477 mg kg⁻¹ f.w. of soil (KQ/PB-52); Mg-60.0-89.0 mg kg⁻¹ f.w. of soil (KQ/PB53); Ca-462.0-619.0 mg kg⁻¹ f.w. of soil (KQ/PB-52); total nitrogen: 23.4-63.5 mg kg⁻¹ f.w. of soil (Chemical and Agricultural Station in Lublin). Cucurbitaceae plants (cucumber) were the preceding crops for each year of the study.

Tomato seedlings were produced in accordance with the generally accepted rules for this vegetable using horticultural soil (peat moss) and quartz sand at a ratio of 2:1 (Welbaum, 2015). Four week old seedlings (at the stage of three true leaves) were planted in a plastic tunnel in the first week of May where the distance between the rows was 0.80 m, and the spacing between the plants in the row was 0.50 m (2.5 plants m²). Before planting the plants in a permanent place, mycorrhizal inoculum was applied using 25-30 spores of MF in physiological saline solution (5 ml) to the

rhizosphere of each seedling (to a depth of 5 cm). In each year of the studies, the field experiment was randomized in a complete block design as a two-factor experiment with five replicates and three treatments. The experimental treatments were: plants inoculated with *C. etunicatum* (CE), plants inoculated with *R. intraradices* (RI), plants without mycorrhizal inoculation as control (C). The above-mentioned treatments were used for each of the tomato cultivars. Black film was used for mulching in the rows in order to protect plants from weeds. Plants were irrigated using drip irrigation depending on their requirements. No fertilization or chemical protection was applied during the vegetation period.

The colonization of roots by the tested mycorrhizal fungi (MF) was measured at the stage of full fructification of the tomato plants (BBCH 89). The evaluation of root colonization (%) by MF was carried out using the Phillips and Hayman method (Phillips and Hayman, 1970). The percentage of root colonization was calculated when five plants were taken from each experimental combination. The lateral roots of these plants were selected, washed under tap water and cut into 0.5 cm fragments. Washing was performed, so that the mycelium and the spores, which are attached to the roots, would not be washed away. After cleaning, the root fragments were transferred into 10% KOH solution and boiled at 90°C for 1 h. After cooling, the root fragments were washed in distilled water and transferred into 1% HCl at 90°C for 20-30 min to acidify the roots. Next, the root fragments were washed in distilled water 2-3 times. They were stained with 0.05% trypan blue solution. The stained root fragments were placed in De-stain solution, then placed on a slide for the observation of the mycelium of MF under the microscope. The colonization of the roots with MF was estimated using the following formula (Phillips and Hayman, 1970) :

$$\text{Root colonization} = \frac{\text{No. of root fragments showing colonization}}{\text{Total number of root fragments observed}} \times 100. \quad (1)$$

In order to measure the leaf nutrient content, samples of tomato leaves were collected at the stage of full fructification of the tomato plants (BBCH 89), from the top shoots (fourth leaf on the shoot top). 50 leaves were randomly selected in each experimental combination. After microwave mineralization of the plant material with HNO₃, the magnesium (Mg), potassium (K) and calcium (Ca) contents were estimated using Flame Atomic Absorption Spectroscopy (FAAS)64. The leaf nitrogen (N) concentration was determined using the Kjeldahl method following the procedures of Sadasivam and Manickam (2005).

The length of the stem and the number of leaves were measured at the beginning of fructification (BBCH 76) and at full fructification of the tomato plants (BBCH 89). The length of the root was measured at the stage of full fructification of the tomato plants (BBCH 89). Five plants from

each experimental combination were chosen randomly for the calculations. The length of the stem and the root (cm) was recorded as the average size obtained from the measurements, which were carried out for each experimental combination. In order to calculate the average number of leaves per plant, the total number of leaves was divided by the number of plants measured in a given experimental combination.

The disease index (*DI*) was determined twice in the growing season: BBCH 76, BBCH 89. These parameters were measured for ten plants from each experimental combination using the authors' own 5-degree scale: 0° – no disease symptoms, 1° – small yellow spots on the leaves covering up to 25% of the leaf area, 2° – yellowish leaves and small necrotic spots on the leaves covering up to 50% of the leaf area, 3° – necrotic spots on the leaves covering an area ranging from 50 to 75% of the leaf area, 4° – extensive necrotic spots on the leaves covering over 75% of the leaf area, with wilting, dying leaves. The values obtained in degrees were converted to the disease index (*DI*, %) according to the Townsend-Heuberger formula (Wezel, 1948):

$$DI = \frac{\sum nv}{NV} \times 100, \quad (2)$$

where: *n* is the number of plants assigned to the class, *v* represents the numeric value of the scale, *N* is the total number of the plants in the replication and *V* is the numeric value of the highest scale.

Tomato fruit were harvested by hand from marked plants during the commercial harvest period (second 10-day period of July until the first 10-day period of October – BBCH 76-88), when the fruit reached the technological maturity stage indicated by the red colour. The harvest took place successively, every 7-14 days, at the phase of technological maturity of the fruit. The yielding parameters were calculated for each plant from the experimental combination on the basis of five replications. The fruit yield is presented in kg m⁻². The total yield included marketable fruit, fruit with disease symptoms and undersized fruit (with a weight of less than 30 g).

The data were analysed using One-way Analysis of Variance (ANOVA) by using SAS Version 9.1 software (SAS Inst., Cary, N.C., USA). Group comparisons were made to determine if the variations among the means of parameters from various treatments were significantly different at a 5% significance level ($p \leq 0.05$).

RESULTS

The inoculation of roots with MF had an influence on the percentage of tomato root colonization. The degree of colonization depended on the species of fungus used for mycorrhization and the study year, but it did not depend on the tomato cultivar. Tomato roots, which were strongly colonized by *C. etunicatum* (GE-49.15%) and *R. intraradices* (RI-46.52%), differed significantly from the control

(C-23.96%) (Fig. 1). The degree of root colonization did not, however, depend on the species of the applied mycorrhizal fungus. The roots of the plants were colonized most in 2015 and least in 2017, and the differences were statistically significant (Fig. 1).

The measurement of the content of macronutrients in tomato leaves allowed for the evaluation of the influence of the tested MF on the extent of nutrient uptake from the soil. The application of *C. etunicatum* and *R. intraradices* within tomato roots resulted in an increase in the mean K content in the leaves of the studied tomato cultivars (CE-4.51 g kg⁻¹ f.w., RI-4.34 g kg⁻¹ f.w.), as compared to the control (C-4.20 g kg⁻¹ f.w.), but they were not statistically

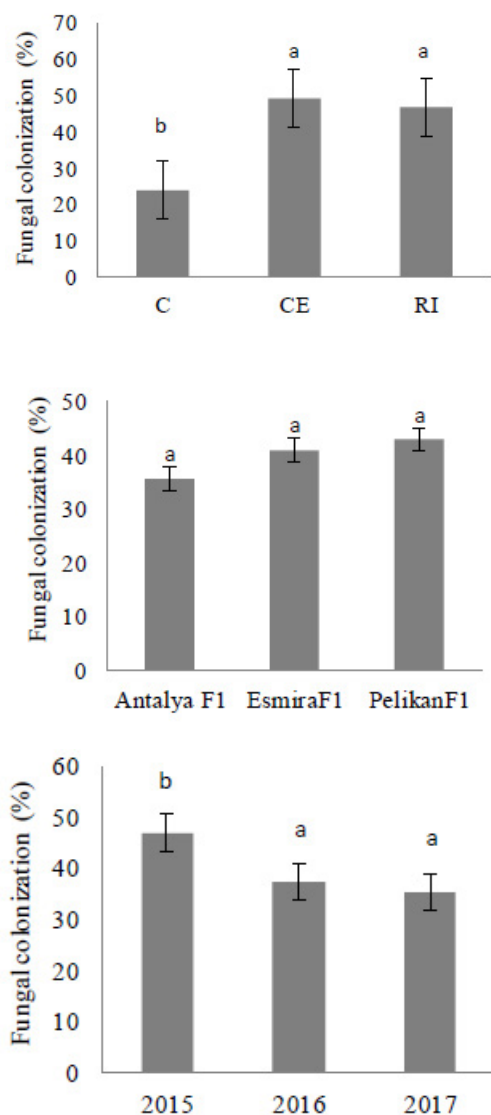


Fig. 1. Colonization of tomato roots by mycorrhizal fungi during the growing season 2015-2017 (%); mean for years, experimental combinations, and cultivars; C – control, CE – *C. etunicatum*, RI – *R. intraradices*; Error bars are the standard error of the means. The letters (a,b...) shown in the figure point to significant differences among the treatments at $p \leq 0.05$ (Tukey's test).

significant differences (Table 1). The content of K in the tomato leaves statistically depended on the cultivar used and the year of the study. Significant differences in the K content of the leaves were observed for cultivars 'Esmira F₁' and 'Pelican F₁', where the content of K was significantly higher after the application of *C. etunicatum* than in the control (Fig. 2).

A high content of Ca in the leaves was observed after the application of *R. intraradices* (RI-5.82 g kg⁻¹ f.w.) and *C. etunicatum* (CE-5.58 g kg⁻¹ f.w.), and the differences were statistically different from the control (C-5.27 g kg⁻¹ f.w.). The mean content of Ca in the tomato leaves also depended on the cultivar. The highest mean content of Ca was found for 'Esmira F₁', while the lowest one occurred for 'Pelikan F₁' (Table 1, Fig. 2).

The leaf K and Ca content during three years of studies was related not only to the presence of MF in the soil but also to the degree of root colonization (Fig. 3). Our results showed a positive linear relationship between the degree to which the roots were colonized by *C. etunicatum* and the mean K leaf content, but a negative correlation between the degree to which the roots were colonized by *R. intraradices* and the mean content of K in the leaves. By contrast, Ca leaf content increased significantly depending on the degree of root colonization by *R. intraradices*, while a very weak relationship was found between the degree of root colonization by *C. etunicatum*, and the mean content of Ca in tomato leaves.

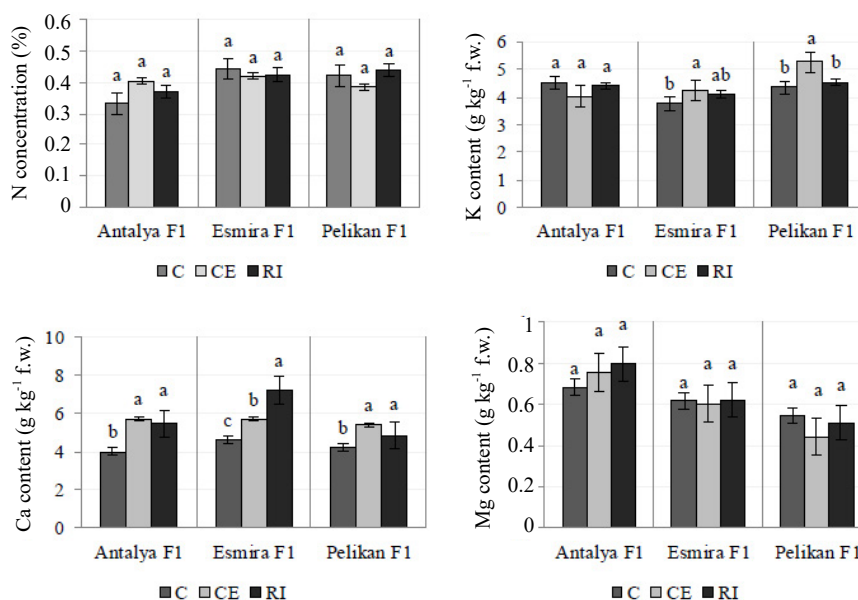
The mean N concentration (%) and mean Mg content in tomato leaves were not significantly affected by the application of the studied MF. On the other hand, the content of N and Mg in the leaves was related to the cultivar and year of cultivation (Table 1).

The data presented in Table 2 indicate that the inoculation of tomato seedlings with MF had a beneficial effect on the growth of the plants (length of stems and roots) although the results obtained were not statistically significant ($p \leq 0.05$). The significant influence of MF on the number of tomato leaves was observed. During the three years of research work, no statistical differences were shown between the stem length of the tomatoes inoculated with MF and the control although the plants inoculated with *C. etunicatum*, *R. intraradices* were longer (respectively, GE-121.95 cm, RI-119.59 cm) than the control plants (C-110.97 cm). The length of the tomato stems depended on the cultivar features and the period of measurement, and not on the species of mycorrhizal fungi. MF application, on the other hand, affected the length of the tomato roots. Plants inoculated with *C. etunicatum* had significantly longer stems (GE-50.95 cm), compared to the roots of the control plants (C-44.67 cm), but they did not differ statistically from the roots of tomatoes inoculated with *R. intraradices* (RI-48.32 cm). The length of the tomato roots differed, depending on the year of cultivation, but this did not depend on the cultivar used.

Table 1. Effects of tomato mycorrhization on leaf nutrient content during the growing season 2015-2017; means for experimental combinations, years and cultivars

Factor	N concentration (%)	Content (g kg ⁻¹ f.w.)			
		K	Ca	Mg	
Experimental combination	Control	0.40 a*	4.20 a*	4.27 a*	0.61 a*
	<i>C. etunicatum</i>	0.40 a	4.51 a	5.58 b	0.60 a
	<i>R. intraradices</i>	0.41 a	4.34 a	5.82 b	0.64 a
	LSD ($\alpha = 0.05$)	0.05	0.40	0.53	0.12
	F ⁰ value	0.239062	1.647761	28.24725	0.357287
	P value	0.787648	0.19575	3.18 10 ⁻¹¹	0.700132
Cultivar	Antalya F ₁	0.37 a*	4.31 a*	5.05 a*	0.75 c*
	Esmira F ₁	0.43 b	4.03 a	5.83 b	0.61 b
	Pelikan F ₁	0.42 b	4.71 b	4.79 a	0.50 a
	LSD ($\alpha = 0.05$)	0.04	0.38	0.60	0.11
	F ⁰ value	5.488003	8.786592	9.683572	13.97932
	P value	0.004957	0.00024	0.000108	2.55 10 ⁻⁶
Year	2015	0.51 b*	3.70 a*	5.53 b*	0.35 a*
	2016	0.36 a	5.09 c	5.44 b	0.67 b
	2017	0.34 a	4.26 b	4.70 a	0.84 c
	LSD ($\alpha = 0.05$)	0.03	0.31	0.59	0.07
	F ⁰ value	108.482	57.70345	6.728275	128.6434
	P value	1.94 10 ⁻³⁰	1.44 10 ⁻¹⁹	0.001567	5.88 10 ⁻³⁴

*Comparison of data within the column marked with an asterisk; values designated with the same letters in columns (a.b.c...) do not statistically differ at $p \leq 0.05$ (Tukey's test).

**Fig. 2.** Effect of MF application on leaf nutrient content in tomato plants during the growing season 2015-2017. Error bars are the standard error of the means. Notes as in Fig. 1. The letters (a,b,c...) shown in the figure point to significant differences among the treatments at $p \leq 0.05$ (Tukey's test).

The application of MF in tomato roots significantly influenced the number of tomato leaves per plant. Tomato plants inoculated with *C. etunicatum* and *R. intraradices* had 18.18-18.9 pcs plant⁻¹ on average, for the studied cultivars, while the control only had 16.38 pcs plant⁻¹ (Table 2). The number of leaves was also related to the cultivar used,

the year of the study and the date of measurement. It was found that in 2016 and 2017, the number of leaves was higher than in 2015. A significant effect of MF on the number of tomato plant leaves was observed in the period of full fructification (22.44 pcs plant⁻¹) as compared to the beginning of the fruiting period (12.73 pcs plant⁻¹).

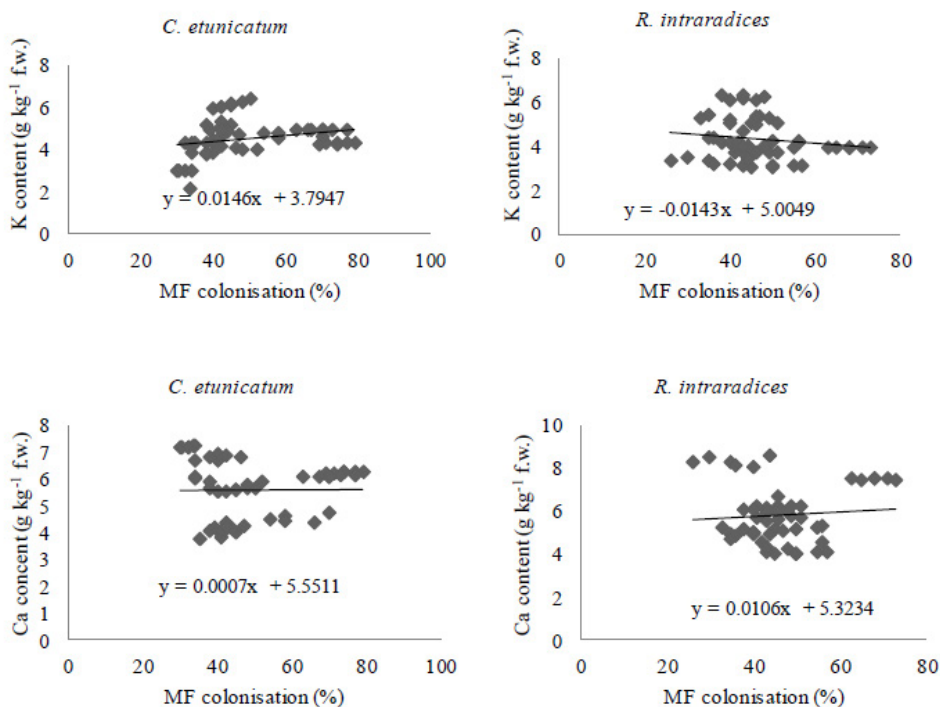


Fig. 3. Relationships between the MF colonization (%) of roots and mean Ca and K leaf content (g kg⁻¹ f.w.); means for combinations, years and cultivars.

Table 2. Effects of tomato mycorrhization on the length of the tomato stem and root (cm plant⁻¹) and on the number of tomato leaves (pcs plant⁻¹) in the growing season in 2015-2017; means for experimental combinations, years and cultivars

	Factor	Length of stem (cm)	Length of root (cm)	Number of leaves (pcs plant ⁻¹)
Experimental combination	Control	110.97 a*	44.67 a*	16.38 a*
	<i>C. etunicatum</i>	121.95 a	50.95 b	18.18 b
	<i>R. intraradices</i>	119.59 a	48.32 ab	18.19 b
	LSD ($\alpha = 0.05$)	11.26	4.80	1.35
	F ⁰ value	2.894229	4.835635	6.58436
	P value	0.056205	0.009148	0.001496
Cultivar	Antalya F ₁	123.11 b*	49.26 a*	18.26 b*
	Esmira F ₁	110.14 a	46.91 a	16.54 a
	Pelikan F ₁	119.26 ab	47.77 a	17.95 b
	LSD ($\alpha = 0.05$)	11.24	4.92	1.35
	F ⁰ value	3.849729	0.651394	5.008774
	P value	0.021875	0.522704	0.006995
Period	BBCH 76	76.35 a*		12.73 a*
	BBCH 89	158.66 b	–	22.44 b
	LSD ($\alpha = 0.05$)	3.36		0.44
	F ⁰ value	2301.452		1831.92
	P value	1.7 10⁻¹⁹⁶		2 10⁻¹⁷⁵
Year	2015	117.90 a*	51.59 b*	16.94 a*
	2016	115.79 a	45.94 a	18.52 b
	2017	118.81 a	46.41 ab	17.29 ab
	LSD ($\alpha = 0.05$)	11.32	4.80	1.36
	F ⁰ value	0.205235	4.768075	4.118654
	P value	0.81452	0.009749	0.016783

*Comparison of data within the column marked with an asterisk; values designated with the same letters in columns (a.b...) do not statistically differ at $p \leq 0.05$ (Tukey's test).

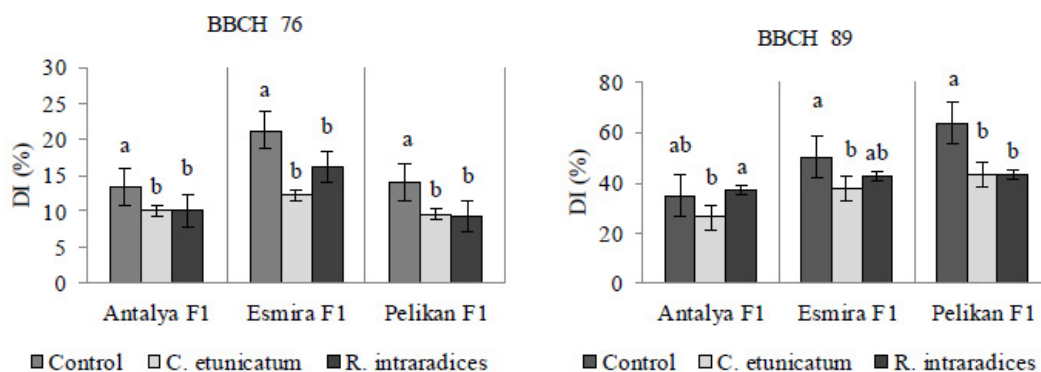


Fig. 4. Effect of mycorrhizal fungi application on the disease index of tomato plants during the growing season 2015-2017 (for BBCH76; BBCH 89). Error bars are standard errors of the means. The letter (a,b,...) shown in the figure point to significant differences among the treatments at $p \leq 0.05$ (Tukey's test).

Table 3. Effects of tomato mycorrhization on fruit yield (kg m^{-2}) in the growing season of 2015-2017; means for experimental combinations, years and cultivars

Factor	Yield (kg m^{-2})			
	Marketable	Diseased fruit	Other	
Experimental combination	Control	10.31 a*	0.78 b*	0.69 a*
	<i>C. etunicatum</i>	10.92 a	0.38 a	0.60 a
	<i>R. intraradices</i>	10.36 a	0.47 a	0.70 a
	LSD ($\alpha=0.05$)	0.93	0.15	0.18
	F ⁰ value	1.485001	22.29628	0.884826
	P value	0.229626	2.91 10^{-9}	0.414808
Cultivar	Antalya F ₁	9.88 a*	0.29 a*	0.42 a*
	Esmira F ₁	11.12 b	0.83 c	0.53 a
	Pelikan F ₁	10.59 ab	0.51 b	1.04 b
	LSD ($\alpha=0.05$)	0.91	0.13	0.14
	F ⁰ value	5.218608	46.78324	65.00117
	P value	0.006381	1.05 10^{-26}	2.34 10^{-21}
Year	2015	11.59 c*	0.46 a*	0.74 b*
	2016	9.45 a	0.64 b	0.53 a
	2017	10.54 b	0.52 ab	0.71 b
	LSD ($\alpha=0.05$)	0.85	0.16	0.18
	F ⁰ value	17.64301	3.571794	4.305712
	P value	1.2 10^{-7}	0.030383	0.015099

*Comparison of data within the column marked with an asterisk; values designated with the same letters in columns (a,b,...) do not statistically differ at $p \leq 0.05$ (Tukey's test).

The studies show a large difference in the degree of plant infection depending on the stage of plant growth and the fungus species used for mycorrhization. The *DI* of the plants was lower at the beginning of tomato fruiting period (from 5.6 to 29.3%) than during the full fructification (from 12.4 to 74.4%) (Fig. 4). The inoculation of tomato plants with MF had an effect on the improvement of the plant health status. The application of mycorrhizal fungi within the rhizosphere of the plants had a significant effect on the decrease in the *DI* values of the plants. The only exception was 'Antalya F₁' inoculated with *R. intraradices*, where the *DI* value was higher than in plants inoculated with *C. etunicatum* and in control plants. The lowest *DI* value was observed for all plants inoculated with *C. etunicatum*, but it

was not statistically lower ($p \leq 0.05$) than for plants inoculated with *R. intraradices* for all cultivars, at different times of observation.

The data presented in Table 3 indicate that the application of MF had no effect on the marketable tomato yield while MF had a significant effect on the diseased fruit yield. Tomato inoculation with MF resulted in an increasing trend in the marketable yield. Inoculated plants showed an increase in the mean yield from 10.36 kg m^{-2} (RI) to 10.92 kg m^{-2} (CE) compared with the control (C), with an average yield of 10.31 kg m^{-2} . The applied MF significantly decreased the yield of diseased fruit. The best results were obtained with the application of *C. etunicatum*, where the mean yield of diseased fruit was 0.38 kg m^{-2} (CE), while for

the control it reached 0.78 kg m⁻² (C). CE and RI decreased the yield of diseased fruit by 51 and 39.7% as compared with the control, respectively. Significant differences in the marketable and diseased yields were also observed between the cultivars and the years of observation (Table 3).

DISCUSSION

The present study shows that the mycorrhizal fungi studied had a beneficial effect on the nutritional status of tomato plants, expressed by the increased content of Ca and K in the leaves. The increased uptake of macro- and microelements such as potassium, nitrogen, calcium and magnesium has also been observed by other researchers (Hart *et al.*, 2015; Wu *et al.*, 2010; Jamiołkowska *et al.*, 2018). An increase of mineral nutrient concentration (P, K, Mg, Fe, Mn, Zn, and Cu) in the leaves was confirmed by Cimen *et al.* (2010) for tomato plants inoculated with AM Fungi. The shoots of the mycorrhizal plant had significantly higher concentrations of P, N, K and Mg but lower Na than those of non-mycorrhizal plants (Abdel-Fattah and Asrar, 2012). Other authors (Maboko *et al.*, 2013) showed no significant concentration of minerals in the leaves of mycorrhizal plants and a lack of increased nutrient uptake by plants that were not inoculated with AM Fungi. The nutrient contents of tomato fruit varied depending on the time of inoculation with AM Fungi. Early inoculation of the plants with mycorrhizal fungi had a positive effect on the quality of the tomato fruit (Nzanza *et al.*, 2012).

An important macronutrient in the development of tomatoes is calcium. Michałojć *et al.* (2015) noted that a higher level of calcium was found in the fruit of tomato plants inoculated with AM Fungi as compared with the fruit from non-mycorrhizal plants. Own research confirms this relationship. Similarly, potassium is the most important nutrient for tomato plants. It determines both the yield and quality of the cultivated plants (Afzal *et al.*, 2015), and in the case of potassium deficiency, plant growth is inhibited. AM Fungi application improves the increase in K content in tomato fruit (Ordookhani *et al.*, 2010). The increased nutrient uptake by AM Fungi may occur through two mechanisms. Mycorrhizal hyphae capture nutrients directly by increasing the absorption by extraradical hyphae, this shortens the transport route of the nutrients from the soil before they reach the roots. Direct uptake and transport of organic and inorganic N, as well as K and Ca to the plant are affected by the extraradical hyphae of AM Fungi (George *et al.*, 1992). The second mechanism responsible for the uptake of K, Ca and Mg by mycorrhizal plants is the improved water uptake, which hastens the flow of these nutrients through the plant roots colonized by AM Fungi (Kothari *et al.*, 1990). Mycorrhizal plants have higher root hydraulic conductivities than non-mycorrhizal plants (Ruiz-Lozano *et al.*, 1996). Higher soluble sugar levels and electrolyte concentrations in mycorrhizal plants are respon-

sible for the improved osmoregulation of these plants (Feng *et al.*, 2002). The degree of root colonization by endomycorrhizal fungi has an influence over the type and quantity of nutrients supplied to the plant. This also causes changes in the quality and quantity of root exudates produced by the mycorrhized plants (Song *et al.*, 2015; Treseder, 2013).

Mycorrhizal fungi participate actively in plant development (Treseder, 2013; Subramanian *et al.*, 2006; Smith and Smith, 2011). Our results showed that the studied MF affected the root length of the investigated tomato cultivars. Where *C. etunicatum* was used, the roots were by 6.28 cm longer than for the control, while in the case of the application of *R. intraradices* the roots of this combination were by 3.65 cm longer as compared with the control roots (Table 2). The results achieved in present study also showed the beneficial effect of MF on the length of the tomato stems. The stems of the mycorrhizal plants were longer than those of the control plants despite a lack of statistical differences. *C. etunicatum* and *R. intraradices* significantly increased the number of leaves, whereas the plants inoculated with MF had, on average, two leaves more on each plant compared with the control (Table 2). Improvement of the vegetative growth of tomato plants may be attributed to the growing conditions and the good condition of the plants, which facilitates the uptake of nutrients as well as improving the photosynthesis and transport of metabolites (Ezzo *et al.*, 2010). Our results are consistent with the findings of many researchers (Oseni *et al.*, 2010; Guru *et al.*, 2011; Tanwar *et al.*, 2013; Castillo *et al.*, 2013). This positive effect may refer to the role of MF in enhancing the uptake of nutrients and successive water retention, which leads to improved plant growth (Treseder, 2013; Wang *et al.*, 2008). The improved growth and the greater number of leaves of the mycorrhizal tomatoes were due to better K absorption by the plants, which was expressed by the increased concentration of K in the leaves (Table 1). Inoculation at the initial stage of plant development can promote AM symbiosis, which leads to the increased growth of plants in the nursery and improved performance after planting in the field. According to Wang *et al.* (2008), the higher values of the growth parameters of AM Fungi inoculated the tomatoes compared to other treatments, which were observed 31 days after plant inoculation. However, other authors show an inhibiting effect of AM Fungi on plant growth (Walling and Zabinski, 2006). This may be due to an increase in the metabolic activity of AM Fungi and a reduction in the carbon transport to the host plant (Smith and Smith, 2011). AM Fungi play different roles in plant growth and this is due to their functional diversity.

During the three-year study, our observations have shown the beneficial effect of *C. etunicatum* and *R. intraradices* on the health status of tomato plants. The severity of the disease, expressed as DI, was statistically dependent on the fungus species used for mycorrhization. The DI of the tomato plants also varied depending on the

stage of plant growth. The potential of AM Fungi to control pathogenic fungi has been clearly described by many researchers (Joseph and Sivaprasad, 2012; Jamiołkowska *et al.*, 2017). The positive effects of AM Fungi inoculation on the health status of plants and on the decrease of pathogen development, such as *Fusarium oxysporum* f. sp. *lycopersici* on tomato, *Phytophthora parasitica*, *Rhizoctonia solani*, *F. oxysporum* f. sp. *cucumerinum*, *Rhizoctonia solani* (Akköprü and Demir, 2005; Pozo *et al.*, 2002; Berta *et al.*, 2005; Hao *et al.*, 2005; Kasiamdari *et al.*, 2002), have been described. Disease inhibition is related to plant mycorrhization, changes occurring in the root system and better absorption of nutrients (Tahat and Kamaruzaman Sijam Othman, 2010).

Own data showed that the best results in the protection of tomato plants against diseases were obtained after the application of *C. etunicatum* (syn. *Glomus etunicatum*), where the value of the disease index (DI) of the studied plants was the lowest among the studied experimental combinations. The positive effect of *Glomus* spp. in plant protection has been described by many authors (Kobra *et al.*, 2009). Manila and Nelson (2014) found that not only the application of one MF species but also their integration has a significant effect on the reduced incidence of wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* in the tomato rhizosphere. In our own studies, interesting results were also obtained with *C. etunicatum*. These results were confirmed by Kobra *et al.* (2009) who observed a low disease index in diseased cotton plants colonized by *G. etunicatum*, whereas the colonization percentage of plant roots was high and therefore relevant to the health of these plants. Own studies showed the high levels of colonization of tomato roots by the mycorrhizal fungus (Fig. 1) studied, which probably have an influence over the low DI of the inoculated plants. The effectiveness of mycorrhizal inoculation and AM Fungi persistence depend on several abiotic and biotic factors in the soil, including the temperature, soil moisture, species of mycorrhizal fungus, time of mycorrhizal inoculation, levels of mycorrhizal inoculum, and also on the potential of the inoculum and the potential of the pathogen (Garmendia *et al.*, 2004; Donkó *et al.*, 2014). Mycorrhizal associations also induce plant resistance to abiotic stresses and soil-borne fungal pathogens (Song *et al.*, 2015; Bi *et al.*, 2007; Jamiołkowska *et al.*, 2018). Mycorrhizal colonization (with *Funneliformis mosseae*) enhances tomato resistance to early blight by the priming a systemic defence response, and the jasmonic acid (JA) signalling pathway is essential for mycorrhiza-primed disease resistance. AM Fungi pre-inoculation has led to significant increases in the activities of β -1, 3-glucanase, chitinase, phenylalanine ammonia-lyase (PAL) and lipoxygenase (LOX) in tomato leaves upon pathogen (*A. alternata*) inoculation (Song *et al.*, 2015).

The positive effects of the mycorrhizal inoculation (CE, RI) of tomato plants were also expressed by the decrease in the yield of the diseased fruit (Table 3). The own studies did not show any significant effect of MF on the marketable yield of the studied tomato cultivars. Similar results were reported by Michałojć *et al.* (2015) who noted no beneficial effect of AM Fungi on tomato yield grown in rockwool and straw. Bosco *et al.* (2007) showed no impact of commercial mycorrhizal formulate on the increase in the total or marketable yields of tomato. This was probably due to the natural organic soil richness. It was also suggested that an improved fruit yield could be related to an increase in pollen quantity and quality in mycorrhizal plants (Subramanian *et al.*, 2006). A significant amount of other data, however, indicates that AM Fungi inoculation improves tomato growth and yield (Candido *et al.*, 2015; Nzanza *et al.*, 2012; Colella *et al.*, 2014). Tomato plants grown in a field and inoculated with a commercial formulation of AM Fungi produced larger inflorescences, as well as a higher number of flowers and a higher total and marketable yield (Conversa *et al.*, 2013). However, a decreased yield of diseased fruit has an indirect influence over the increase of the marketable fruit yield, which is expressed by a qualitatively better yield, this was proven in own studies. The greatest effect on the size of tomato yield was exerted by the cultivar as well as the conditions of the cultivation, and not by the application of mycorrhizal fungi.

CONCLUSIONS

1. The application of MF to the rhizosphere of tomato plants (*C. etunicatum* and *R. intraradices*) had a positive effect on the uptake of K and Ca by the plants, improved their growth rate and also reduced the disease index of the plants.
2. However, the treatments did not significantly increase of the marketable yield of tomatoes, but rather they decreased the yield of the diseased fruit.
3. A better uptake of macroelements from the soil (higher leaf K and Ca content) plays an important role in the yield formation and development of cultivated plants.
4. The results showed that there were significant relationships between the leaf macronutrient content and root colonization by MF, which was reflected by the improved growth rate of the plants and in the decrease of their infection rate with the pathogenic factors.
5. Among the MF species studied, improved results were obtained with the application of *C. etunicatum* as compared with *R. intraradices* for all examined features.
6. In general, our findings indicated that mycorrhizal inoculation contributes to the improved growth of tomato hybrids, it improved the health status of the plants and can be used as a safe and alternative method in ecological production.

ACKNOWLEDGEMENTS

We would like to thank the Department of Agricultural Microbiology of the Institute of Soil Science and Plant Cultivation – National Research Institute in Puławy in Poland for providing the mycorrhizal fungi spores used in the experiment.

Conflict of interest: The Authors do not declare conflict of interest.

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