ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, online first, 1–11

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2026.5608

RESEARCH PAPER

Received: 30.09.2025
Accepted: 19.03.2026
Online first: 13.05.2026

INFLUENCE OF MYCORRHIZAL FUNGI ON PLANT GROWTH AND RHIZOSPHERE SOIL MICROBIOME OF TOMATO (*Solanum lycopersicum* L.)

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ABSTRACT

Mycorrhizal fungi increase plant resistance to stress factors such as drought, high or low temperatures, acidification, soil contamination with heavy metals, and the presence of soil pathogens. The aim of the study was to determine the effect of mycorrhizal inoculum on plant growth and the microbiome of the rhizosphere soil of tomato. The experiment was established in the greenhouse of the Felin Experimental Farm at the University of Life Sciences in Lublin. The research material consisted of tomato plants (*Solanum lycopersicum* L.) Lubań cv. The mycorrhizal inoculum Endo-VAM from Mykoflor (Końskowola, Poland) was used for mycorrhization of tomato seedlings. It contains spores and live mycelium of the following species: *Glomus intraradices*, *G. mosseae*, *G. claroideum*, *G. etunicatum*, *Gigaspora margerita*, and *Entrophospora* spp. Non-mycorrhized tomato seedlings served as a control. Six weeks after the application of the mycorrhizal inoculum, mycological analysis of tomato roots and microbiological analysis of rhizosphere soil were performed, and plant height, stem base diameter, fresh and dry root weight were determined. The roots of mycorrhized plants were found to be more abundantly colonized by fungi, including antagonistic species, than the roots of non-mycorrhized plants. The total number of bacteria, including *Pseudomonas* and *Bacillus* genera, and the total number of fungi isolated from the rhizosphere of mycorrhized plants were significantly lower than in the rhizosphere of control plants. Analysis of morphological parameters of tomato plants demonstrated a beneficial effect of the mycorrhizal inoculum on the growth of fresh and dry root weight, but did not demonstrate a significant effect of mycorrhiza on plant height or tomato stem base diameter.

Keywords: tomato, mycorrhiza, rhizosphere microbiota, SPAD, total chlorophyll

INTRODUCTION

Tomato is one of the most important vegetable crops worldwide, not only because of its economic importance but also due to the high nutritional value of its fruits [Yang et al. 2023]. Universally consumed tomato fruits provide numerous health benefits, which contribute to a healthy, balanced diet. Tomato fruits contain vitamins A, B, and C, as well as high levels of lycopene and carotenoids, which help protect against various lifestyle-related diseases [Kaboré et al. 2022, Lee et al. 2023, Li et al. 2024]. Tomato fruits are also used in the prevention of neurodegenerative diseases, reducing the incidence of certain chronic conditions. Tomatoes, as an important source of potassium, phosphorus, magnesium, and iron, are essential for proper nerve and muscle function [Raiola et al. 2014, Shah et al. 2021]. They are rich in minerals, essential amino acids, sugars, and dietary fiber. In addition to these

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nutrients, they also contain β-carotene and lycopene pigments [Natali et al. 2025]. Tomato cultivation is exposed to many adverse environmental factors, including pathogens. Many agrochemicals, such as mineral fertilizers and pesticides, are still used in tomato cultivation.

Although chemical horticulture is effective in crop protection, it also contributes to environmental pollution. The need to reduce synthetic fertilization and protect the environment has led to the development of formulations, whose main active components are beneficial microorganisms. The living organisms in biopreparations utilize various mechanisms of action and can affect the soil, plants, or both concurrently. The purpose of such formulas is to improve plant nutrient uptake, reduce the negative effects of abiotic environmental factors, combat phytopathogens, and promote plant growth and development. The main components of such preparations are bacteria and fungi, often isolated from the natural environment, frequently directly from the soil. Examples of such microorganisms are mycorrhizal fungi, which due to their multi-level interactions with plants are increasingly used as potential biocontrol agents [Głuszek et al. 2008]. Mycorrhiza is a strong symbiotic association between soil fungi and the fine plant roots. Structures formed through mycorrhiza benefit both partners, as the fungus receives organic compounds produced by the plant during photosynthesis, while the plant gains organic compounds and water. Mycorrhizal fungi are rarely specific to a single host, meaning that one species can colonize multiple plant species. Arbuscular mycorrhizal fungi (AMF), primarily from the phylum *Glomeromycota*, can protect plants from the harmful effects of abiotic stressors such as drought and heavy metals. The drought tolerance of mycorrhizal plants depends on the AMF species colonizing their roots. Mycorrhizal fungi are believed to modify the growth of aerial plant parts under drought stress by affecting complex plant responses to water deficiency, including phosphorus and potassium uptake, root respiration, photosynthesis, transpiration, and leaf osmotic potential [Głuszek et al. 2008, Jamiołkowska et al. 2018, 2020a]. Mitigation of heavy metal toxicity may result from reduced uptake by plants, either through accumulation in the extraradical mycelium or through changes in metal solubility. Mycorrhizal fungi have also developed various mechanisms to protect plant roots from pathogens. They colonize the root surface and compete with pathogenic microorganisms for nutrients produced by the plant. They stimulate the plant to produce and secrete defense-related compounds [Ruiz-Lozano et al. 2016]. In addition to the benefits conferred to plants, mycorrhizal fungi also contribute to the improvement of soil structure. These fungi form soil aggregates, which positively affect soil quality and plant health. Apart from these benefits, mycorrhiza increases plant resistance to diseases [Pozo and Azcón-Aguilar 2007]. Mycorrhizal fungi share certain traits with biotrophic pathogens and can trigger plant defense responses during the initial stages of symbiosis [Jung et al. 2012, Song et al. 2015]. For colonization to be successful, the fungus must manage these responses and actively modulate plant reactions – a phenomenon known as "priming". Stimulating plant defenses plays a central role in mycorrhiza-induced resistance [Fiorilli et al. 2024]. Gallou et al. [2011] discovered that mycorrhizal colonization increases tomato resistance to late blight by inducing a systemic defense response, and the jasmonic acid (JA) signaling pathway is essential for mycorrhiza-induced disease resistance. Drought tolerance in mycorrhizal plants depends on the species of arbuscular mycorrhizal fungi (AMF) colonizing their roots. These fungi are believed to modify the growth of aerial plant parts under drought stress by influencing complex plant responses to water deficit, including phosphorus and potassium uptake, root respiration, photosynthesis, transpiration, and leaf osmotic potential.

The aim of this study was to determine the effect of a mycorrhizal inoculant on selected plant morpho-physiological parameters, the rhizosphere soil microbiome, and the diversity of fungi colonizing tomato roots.

MATERIALS AND METHODS

Pot experiment

The experiment was conducted in the greenhouse of the Felin Experimental Farm at the University of Life Sciences in Lublin. The plant material consisted of tomato (*Solanum lycopersicum* L.) plants of the cultivar Lubań (PlantiCo – Hodowla i Nasiennictwo Ogrodnicze Zielonki sp. z o.o., Poland). Tomato seedlings were prepared following the standard procedures commonly used for this vegetable (Wysocka-Owczarek 2010). The three-week-old tomato seedlings were transplanted in the first ten days of May into 1-liter plastic pots filled with non-strilized Sterlux horticultural substrate (Agaris, Poland). The substrate used for experiment was based on high-moor peat and enriched with a multi-component NPK + Mg fertiliser (14–16–18) at a rate of 0.6 kg m^{-3}, with a pH of 5.5–6.5.

One plant was transplanted into each pot. To each pot partially filled with horticultural substrate 0.5 g of MF inoculum mixed with 30 ml of semi-liquid potato dextrose agar (PDA, Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was added. Then, tomato seedlings were planted and supplemented with soil substrate. The

tomato seedlings were mycorrhized using the Endo-VAM mycorrhizal inoculum (Mykoflor, Końskowola, Poland) containing oospores and live mycelium of *Glomus intraradices*, *G. mosseae*, *G. claroideum*, *G. etunicatum*, *Gigaspora margerita*, and *Entrophospora* sp. The control group consisted of non-mycorrhized plants. For each experimental treatment, 25 plants were prepared (5 plants in 5 replicates). Plants were grown in pots, on cultivation tables over a capillary mat watered once a day for 2 minutes. The average temperature in the facility during the experiment was 23°C, with air humidity ranging from 30 to 35%. The facility was ventilated automatically. The pot experiment was carried out for 6 weeks.

Assessment of root colonization by mycorrhizal fungi. To assess the degree of root colonization by mycorrhizal fungi, 10 root hairs were collected from 10 randomly selected plants (100 root fragments per experimental treatment), at the end of the pot experiment (i.e. 6 weeks after inoculation of the rhizosphere soil with the mycorrhizal inoculum). The collected root fragments were stained according to the method developed at the Department of Microbiology and Rhizosphere of the Institute of Horticulture in Skierniewice. Microscopic slides were prepared from these roots by selecting 50 one-cm-long fragments from each experimental combination. The fragments were arranged in parallel on a glycerol-coated slide and gently pressed with a cover slip. The histological preparations were examined using a Nikon 50i microscope (40× objective magnification), and photographic documentation of mycorrhizal structures was made. The degree of tomato root colonization by arbuscular mycorrhizal fungi was assessed using the Trouvelot (1986) method. Based on the obtained results, mycorrhizal frequency (F%) and root colonization intensity (M%, m%) were calculated using the MYCOCALC software, available online at: https://www2.dijon.inrae.fr/mychintec/Mycocalc-prg/download.html.

Microbiological analysis of rhizosphere soil. The experiment determined the total number of fungi and bacteria, as well as the bacterial count of genera *Pseudomonas* and *Bacillus* in the rhizosphere soil of tomato. Rhizosphere soil was collected six weeks after inoculating the plants with the mycorrhizal fungi to obtain a representative composite sample for each experimental combination. 5 g of rhizosphere soil was collected from each pot and then mixed (pooled) within the experimental combination. Then 1 g of soil was taken and dried from each pooled sample per combination. After drying, 0.7 g of soil was obtained for the control (C) and 0.8 g for the mycorrhizal treatment (MF). Microbiological analysis was performed on 10 g of rhizosphere soil from each experimental combination. Each soil sample was mixed with 9 ml of sterile distilled water. Subsequently, serial dilutions of the soil solution were prepared. Appropriate dilutions of the soil solutions were plated onto culture media in Petri dishes. For each experimental combination and microbial group, 9 replicates (9 Petri dishes) were prepared with the culture media described above, onto which dilutions of the soil suspension were plated according to the method of Patkowska and Konopiński [2014]. For the isolation of total fungi, Martin's medium was used, containing 20 g of agar, 5 g of peptone, 10 g of dextrose, 1 g of KH_2PO_4, and 0.5 g of $MgSO_4 \cdot 7H_2O$, supplemented with streptomycin and Bengal rose. Streptomycin and Bengal rose were mixed with Martin's liquid medium after sterilization in an autoclave, before pouring the medium into Petri dishes. For the isolation of total bacteria, nutrient agar supplemented with glucose was used (Difco, Becton, Dickinson and Company, Sparks, MD, USA). For the isolation of bacteria from the genus *Pseudomonas*, Pseudomonas Agar F (Difco, Becton, Dickinson and Company, Sparks, MD, USA) supplemented with penicillin and nystatin was applied. Penicillin and nystatin were mixed with liquid medium Pseudomonas Agar F medium after sterilization in an autoclave, before pouring the medium into Petri dishes. Triptic Soy Agar (Difco, Becton, Dickinson and Company, Sparks, MD, USA) was used to isolate bacteria from the genus *Bacillus* [Jamiołkowska et al. 2020b].

Root mycological analysis. Mycological analysis of tomato roots was conducted six weeks after mycorrhizal inoculation (beginning of flowering, BBCH 62), following the method described by Jamiołkowska [2007]. For the analysis, roots were collected from 10 plants per experimental combination. The roots were cleaned of substrate residues and rinsed under running water for 20 minutes. They were then surface-sterilized in a 10% sodium hypochlorite solution. The roots were cut into 0.5-cm fragments, and 10 fragments were placed on each Petri dish. Glucose-potato agar (PDA, Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used in the study. Twenty dishes were prepared for each experimental combination. The dishes with root fragments were incubated at 24°C for 7 days. Subsequently, the resulting fungal colonies were transferred onto potato-dextrose agar (PDA, Difco, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and identified to the genus level based on microscopic characteristics and available mycological monographs.

Assessment of root fresh and dry weight. The analysis was conducted on 15 roots from plants in each experimental combination. The fresh root mass was expressed as g FW^{-1}. The roots were then dried for 7 days in a ventilated room at 23–25°C and weighed, and the results were expressed as g DW^{-1}.

Plant height and stem base thickness measurements. The assessment of plant height and stem base diameter was performed 6 weeks after mycorrhizal inoculation of the plants (beginning of the flowering stage – BBCH 62). The height of the aerial plant part (cm) was measured from the soil surface to the plant apex for each plant in the combination. Stem thickness (mm) was measured at the base of each plant in the experimental combination using a caliper (Limit CDN-NT IP67).

Leaf greenness (SPAD) and total chlorophyll measurements. Leaf greenness of tomato plants was measured during the experiment using a SPAD-502 Plus chlorophyll meter (Konica Minolta). Non-invasive measurements were taken at three time points, at 7-day intervals, starting 10 days after mycorrhizal inoculation (15 May 2024, 22 May 2024, and 29 May 2024). The measurements were conducted on the third fully developed leaf from the apical meristem. Based on the measurements, SPAD values were converted to total chlorophyll content using the formula provided by Shenker et al. [2004] for tomato plants:

$$\text{Chlorophyll} = \frac{\text{SPAD} - 6.6}{27.3} \ (\text{mg g}^{-1}\,\text{FW})$$

Statistical analysis

The collected data were analyzed using Statistica software version 13.3 (1984–2017 TIBCO Software INC, Palo Alto, CA, USA). The normality of data distribution was tested using the Shapiro-Wilk test. A one-way analysis of variance (ANOVA) was conducted, and the significance of differences was assessed using the Tukey post hoc test and Kruskal-Wallis test at a significance level of $p = 0.05$.

RESULTS

Evaluation of root colonization by mycorrhizal fungi. Laboratory assessment of tomato roots showed that roots of mycorrhized plants (MF) were colonized by mycorrhizal fungal structures at 30%, whereas mycorrhizal frequency in control roots (C) was 18.89% (Table 1, Figure 1). Relative mycorrhizal intensity (M) was higher in the mycorrhizal treatment (MF – 2.59%) than in the control (C – 1.7%). No arbuscules (A) were observed in the roots of either mycorrhized or control plants. No significant differences were observed in absolute mycorrhizal intensity between the experimental combinations (m) – as shown in Table 1.

Table 1. Colonization of tomato roots by mycorrhizal fungi

Experimental treatment	F (%)	M (%)	m (%)	A (%)
MF	30.0[b]	2.59[a]	8.68[a]	0
C	18.89[a]	1.70[a]	8.92[a]	0

MF – mycorrhized plants, C – control plants, F – mycorrhiza frequency, M – relative mycorrhiza intensity, m – absolute mycorrhiza intensity (refers to fragments where any mycorrhizal colonization was observed), A – absolute arbuscule abundance (refers to fragments where arbuscules were detected); [a, b] – values in the same column marked with the same letter do not differ significantly at $p \leq 0.05$

Microbiological analysis of rhizosphere soil. The laboratory analysis revealed variation in the quantitative and qualitative composition of rhizosphere microorganisms. Mycorrhization of the plants significantly reduced the total number of bacteria and fungi in the tomato rhizosphere. The bacterial population in the rhizosphere of mycorrhized plants was 32.2×10^6 CFU g^{-1} DW soil, whereas it was twice as high in the control (72.4×10^6 CFU g^{-1} DW) soil (Table 2). The total number of fungi in the rhizosphere of mycorrhized plants was also significantly lower (3.1×10^5 CFU g^{-1} DW soil) than in the control (8.8×10^5 CFU g^{-1} DW soil) – as shown in Table 2. The mycorrhizal inoculum inhibited the growth of *Bacillus* sp. and *Pseudomonas* sp. in the tomato rhizosphere soil. The population of *Pseudomonas* sp. in the control was nearly twice as high (12.7×10^6 CFU g^{-1} DW soil) as in the rhizosphere soil of mycorrhized tomato plants (6.6×10^6 CFU g^{-1} DW soil). A lower number of bacteria from the genus *Bacillus* was also detected in the rhizosphere soil, but this difference was not statistically significant (Table 2).

Figure 1. Mycelium (m) and vesicles (vs) of mycorrhizal fungi in tomato roots; MF – mycorrhized plants, C – control plants; microscope objective magnification: 40×

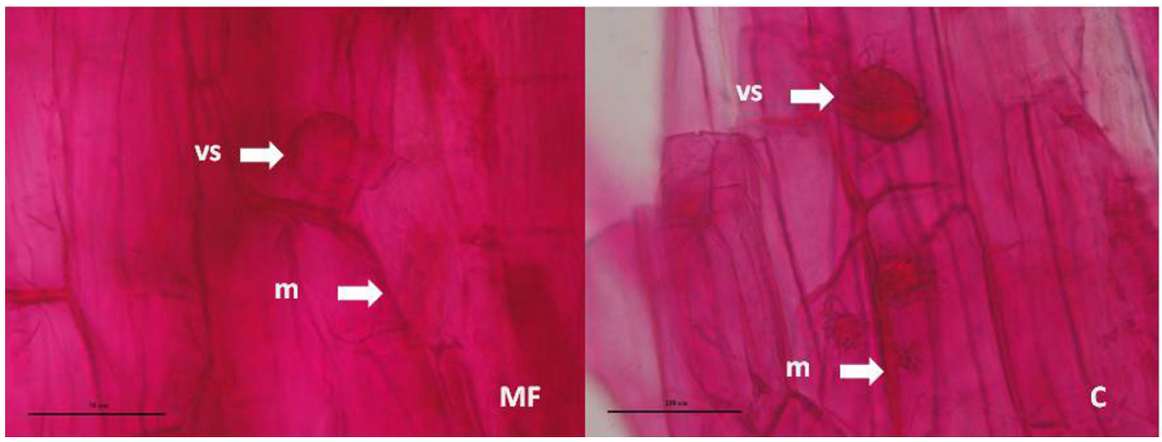


Table 2. Number of bacteria and fungi isolated from tomato rhizosphere soil

Experimental treatment	Total bacterial count(10^6 CFU g^{-1} DW soil) ±SD	*Bacillus* sp. count (10^6 CFU g^{-1} DW soil) ±SD	*Pseudomonas* sp. count (10^6 CFU g^{-1} DW soil) ±SD	Total fungal count (10^5 CFU g^{-1} DW soil) ±SD
MF	32.2 ±2.75[b]	1.5 ±0.21[a]	6.6 ±0.96[b]	6.6 ±1.60[b]
C	72.4 ±18.74[a]	2.37 ±0.77[a]	12.7 ±2.40[a]	8.8 ±1.08[a]

MF – mycorrhized plants, C – control plants, [a, b] – values in the same column marked with the same letter do not differ significantly at $p \leq 0.05$

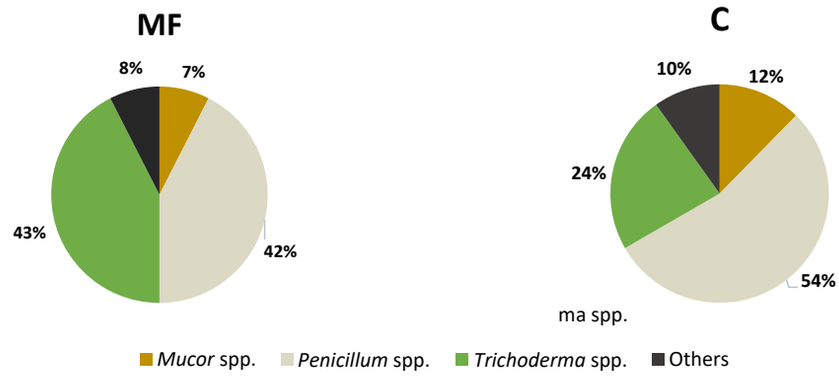
Fungal diversity colonizing tomato roots. The mycological analysis of tomato roots identified a total of 160 fungal colonies belonging to 10 genera (Table 3). The most abundant genera were *Penicillium* spp., *Trichoderma* spp., and *Mucor* spp. *Trichoderma* (33.1%), *Penicillium* (48.8%), and *Mucor* (10.0%) accounted for the largest proportions of the overall fungal composition (Table 3). *Penicillium* spp. were the most frequently isolated fungi in the control (C; 54.0%) – as shown in Figure 2. Meanwhile, the genus *Trichoderma* was dominant (43.0%) in the mycorrhizal treatment (MF) – as shown in Table 3 and Figure 2. The presence of mycorrhizal fungi led to a reduction in the abundance of other genera (including *Penicillium* spp. and *Mucor* spp.) in favor of the development of antagonistic *Trichoderma* spp. fungi (Figure 2).

Table 3. Diversity of fungi colonizing tomato roots

Fungal genus	Colony number		Total	Percentage (%)
	MF	C		
Alternaria spp.	1	1	2	1.3
Aspergillus sp.	–	1	1	0.6
Cladosporium sp.	1	1	2	1.3
Colletotrichum sp.	1	–	1	0.6
Humicola spp.	1	1	2	1.3
Mucor spp.	6	10	16	10.0
Penicillium spp.	34	44	78	48.8
Rhizopus sp.	–	1	1	0.6
Trichoderma spp.	34	19	53	33.1
Zygorhynchus sp.	1	3	4	2.4
Total	79	81	160	100

MF – mycorrhized plants, C – control plants

Figure 2. Percentage of selected fungal genera colonizing tomato roots; MF – mycorrhized plants, C – control plants



Plant height and stem base thickness. The study did not reveal any significant differences between the experimental combinations. Control plants were taller (101.14 cm) than mycorrhized plants (96.62 cm), but differences were not statistically significant. Similar trends were observed for stem base thickness, with control plants having thicker stems than mycorrhized tomatoes (Table 4).

Table 4. Mean plant height (cm) and stem base diameter of tomato plants (mm)

Experimental treatment	Plant height (cm) ±SD	Stem base diameter (mm) ±SD	Fresh root weight (g) ±SD	Dry root weight (g) ±SD
MF	96.62 ±6.48[a]	9.15 ±0.27[a]	41.67 ±8.28[a]	5.9 ±1.65[a]
C	101.14 ±8.80[a]	9.25 ±0.89[a]	35.77 ±4.71[a]	5.01 ±0.64[a]

MF – mycorrhized plants, C – control plants, [a, b] – values in the same column marked with the same letter do not differ significantly at $p \leq 0.05$

Fresh and dry root weight. The highest fresh root weight was observed in mycorrhized plants, with an increase of 16.5% compared to the control. Similarly, the highest dry root weight was recorded in mycorrhized plants, showing a 17.7% increase compared to the control. The recorded increase in fresh and dry weight was not statistically significant (Table 4). The results, however, indicated an increasing trend in this trait following mycorrhizal inoculation (Table 4).

Leaf greenness index and total chlorophyll content. The tomato leaf greenness index showed an increasing trend during plant development (Table 5). The highest SPAD value was recorded at the third measurement for mycorrhized tomato leaves (40.9), but it was not significantly higher compared to the control plants. Similar increasing trends in SPAD values were observed at the other time points, with mycorrhized plants showing higher SPAD values than the control plants. These differences, however, were not statistically significant (Table 5). Tomato leaves also showed variation in total chlorophyll content depending on the experimental combination, with the highest values in plants with mycorrhizal inoculum, although the difference from the control was not significant (Table 5).

Table 5. Leaf greenness index (SPAD) and total chlorophyll content in tomato leaves (mg g^{-1} FW)

Measurement	I		II		III	
Experimental treatment	C	MF	C	MF	C	MF
SPAD ±SD	34.21 ±3.20[a]	36.43 ±4.69[a]	35.41 ±3.72[a]	36.69 ±3.55[a]	40.18 ±3.43[a]	40.9 ±2.60[a]
Chlorophyll (mg g^{-1} FW) ±SD	1.01 ±0.12[a]	1.09 ±0.17[a]	1.06 ±0.14[a]	1.10 ±0.13[a]	1.23 ±0.13[a]	1.26 ±0.10[a]

C – control plants, MF – mycorrhizal plants; [a, b] – values within rows for each measurement date followed by the same letter do not differ significantly at $p \leq 0.05$

DISCUSSION

Tomato is a species with a high capacity for forming mycorrhizal symbiosis [Pokluda et al. 2021]. Many authors have confirmed the positive effect of mycorrhizal fungi on tomato plants [Leventis 2021, Felföldi et al. 2022, Singh et al. 2024, Zhang et al. 2024]. Plants that interact with mycorrhizal fungi exhibit increased resistance to adverse environmental conditions [Delaeter et al. 2024]. It has been shown that arbuscular mycorrhizal fungi (AMF) protect plants against the negative effects of biotic and abiotic factors, including drought. Through the symbiosis with plants, they increase gas exchange, leaf water content, stomatal conductance, and plant transpiration rate [Morte et al. 2000, Mena-Violante et al. 2006]. AMF maintain cell membrane integrity, improve nutrient and water uptake, and protect the photosynthetic apparatus from drought-induced oxidative stress. They enhance photosynthetic efficiency, enable the accumulation of hormones and phenols, and reduce the buildup of reactive oxygen species (ROS) by increasing antioxidant activity and gene expression [Tang et al. 2022].

Singh et al. [2024] demonstrated the beneficial effects of mycorrhizal fungi on tomato growth parameters, including plant height, stem diameter, leaf number and area, aerial organ weight, as well as root weight and volume, compared with non-inoculated plants. Our study confirmed the positive effect of a multi-component mycorrhizal inoculum on the fresh and dry root weight of tomatoes, as well as on total chlorophyll content in the leaves of mycorrhized plants. Felföldi et al. [2022] demonstrated that mycorrhizal inoculation improved tomato plant growth, yield, and fruit quality parameters, highlighting the beneficial effect of mycorrhiza on water and mineral uptake from the soil. In the present study, however, mycorrhization had no significant effect on plant height or stem base thickness. This can be explained by the fact that measurements were taken six weeks after plant inoculation, when the effect of mycorrhization was not yet apparent in morphological traits. Hart and Reader [2002] reported that the average time for root colonization, i.e., from inoculation to initial colonization, ranged from 1 to 3 weeks for *Glomus* spp., and from 6 to 8 weeks for *Gigaspora* spp. Smith and Smith [2011] have emphasized that the use of arbuscular mycorrhizal fungi does not always result in a significant increase in plant vegetative biomass. Jamiołkowska et al. [2020b] demonstrated that mycorrhization of tomato roots with *Claroideoglomus etunicatum* and *Rhizophagus intraradices* improved plant uptake of certain macro- and micronutrients (Ca, K), and the root length of tomato plants, particularly those treated with *Claroideoglomus etunicatum*, was significantly greater compared to the control. The authors found out that inoculation of tomato roots with both mycorrhizal fungal strains significantly increased the number of leaves and improved plant health. Tomato yield was not significantly affected by mycorrhization; however, *Claroideoglomus etunicatum* reduced the number of diseased fruits more effectively than *Rhizophagus intraradices* compared to the control [Jamiołkowska et al. 2020b].

The current study showed that inoculation with a multi-component mycorrhizal inoculum modulated the microbiota of tomato rhizosphere soil. Mycorrhizal fungi reduced the total number of bacteria and fungi in the tomato rhizosphere, including antagonistic bacteria from the genera *Bacillus* and *Pseudomonas*. Similar results were obtained by Jamiołkowska et al. [2020b], who reported a reduction in the total number of bacteria, including *Bacillus* sp. and *Pseudomonas* sp., as well as a decrease in the total number of fungi in the rhizosphere soil of sweet pepper inoculated with mycorrhizal inoculum. In our experiment, a higher number of *Trichoderma* spp. fungi was found on the roots of plants in combination with MF. This phenomenon can be explained by the changing composition of root exudates of mycorrhizal plants. Because Mycorrhizal fungi influence root exudates, modify the environmental conditions for microbial growth in the rhizosphere, and thereby alter the composition of soil microbiota [Amer and Abou-el-Seoud 2008, Bücking et al. 2008, Al-Askar and Rashad 2010, Zarea et al. 2011]. Root border cells (RBCs) also play an active role in this process by secreting metabolites into the environment, shaping the populations of microorganisms colonizing the root zone, and participating in interactions between plants and soil microorganisms [Jaroszuk-Ściseł et al. 2009]. This phenomenon is particularly important for stimulating the activity of microorganisms antagonistic to soil pathogens, promoting the growth of microorganisms such as: *Mortierella*, *Pseudogymnoascus*, *Mucor*, *Trichoderma*, *Conocybe*, *Pseudaleuria*, and *Acremonium* [Jamiołkowska et al. 2020b].

Mycological analysis of tomato roots showed that the number of antagonistic fungi of the genus *Trichoderma* spp. was higher in the roots of mycorrhized plants than in those of control plants. Fungi of the genus *Trichoderma* benefit plants by suppressing pathogenic agents. They are strong competitors and exhibit both antibiotic and parasitic activity against phytopathogens. They grow rapidly in the soil and suppress other microorganisms [Błaszczyk et al. 2014]. Enzymes produced by *Trichoderma* spp. (including cellulases, hemicellulases, chitinases, and glucanases) facilitate the degradation of pathogen cell walls [Chen et al. 2025]. It can be assumed that myc-

orrhizal fungi also have a significant impact on the fungal mycobiota of tomato roots inoculated with mycorrhizal fungi. Al-Hmoud and Al-Momany [2015] demonstrated that mycorrhizal fungi reduce the number of pathogens through competition for nutrients, and the application of the mycorrhizal fungus *Glomus intraradices* significantly decreased *Fusarium* spp. infections in the studied plants by promoting plant growth and increasing root volume. The present study confirmed these findings, showing a significantly higher abundance of *Trichoderma* fungi on the roots of mycorrhized plants. Mycorrhizal fungi also improve plant resistance to diseases, in part by regulating changes in root anatomical structure [Jamiołkowska et al. 2020a, Weng et al. 2022]. The most important elements of this phenomenon include accelerated lignification of the cell wall and an increased number of cell layers, formation of hyphal networks and a pectic nodule structure in the root epidermis, callose deposition, and production of hydroxyproline-rich glycoproteins (structural components of the cell wall) [Scervino et al. 2007, Balestrini and Bonfante 2014, Basyal and Emery 2021]. Fujita et al. [2022], using molecular analyses, demonstrated the efficacy of a mycorrhizal inoculum based on *Gigaspora margarita* in protecting tomato plants against *Botrytis cinerea* and *Pseudomonas syringae* pv. tomato. Defense-related genes associated with salicylic acid (SA) and jasmonic acid (JA) were expressed more rapidly and strongly in tomato plants colonized by *G. margarita* compared to control plants, as the plant's immune system was stimulated by mycorrhizal colonization. Resistance induced by this mechanism was effective against both fungal and bacterial pathogens.

The effectiveness of root colonization by mycorrhizal fungi depends on multiple factors, including the type of mycorrhizal inoculum and environmental conditions such as light availability, pH, soil fertility, and moisture [Jamiołkowska et al. 2018, Derkowska et al. 2024]. Literature indicates significant differences in the colonization rate between different species of arbuscular mycorrhizal fungi, highlighting consistent trends characteristic of their taxonomic family [Hart and Reader 2002]. Fungi of the genus *Gigaspora* (family: *Gigasporaceae*), despite their low colonization rate and limited internal root colonization, tend to form extensive, dense, and robust hyphal networks on the root surface [Caccia et al. 2024]. In contrast, fungi of the genus *Glomus* (family: *Glomaceae*) are characterized by a rapid colonization rate, but they produce a more dispersed mycelium with finer hyphae [Säle et al. 2021]. Simultaneously, previous studies have emphasized the need for the coexistence of these mycorrhizal genera to achieve improved effects [Hart and Reader 2002]. Subramanian et al. [2006] demonstrated that the inoculation of tomato seedlings with *Rhizophagus intraradices* increased root colonization by up to 48%. The present study indicates that the applied mycorrhizal inoculum resulted in a colonization frequency of 30%, over one-third higher than in the control combination. The mycorrhizal fungal species and the colonization time also play a significant role in the process of plant root colonization [Hart and Reader 2002].

The study also demonstrated the effect of plant mycorrhization on chlorophyll content in tomato leaves, a important indicator of plant physiological status [Caradonia et al. 2019]. Literature confirms a direct relationship between the chlorophyll content in the plant and the SPAD parameter values defined as a measure of "leaf greenness" [Shenker et al. 2004]. The results showed that leaves of mycorrhized tomato plants had slightly higher chlorophyll content than the control leaves. Although these differences were not statistically significant, it can be assumed that mycorrhization had a positive effect on photosynthesis. Similar experiments were conducted by Fracasso et al. [2020], who confirmed a positive correlation between SPAD values and photosynthesis rate. Similarly, Majkowska-Gadomska et al. [2016] showed that the mycorrhizal inoculum improved the nutritional status of tomato plants determined based on the leaf greenness index (SPAD). The higher photosynthetic efficiency in mycorrhized plants results from an increased density of stomata in the leaves and elevated levels of photosynthetic pigments (increased concentrations of chlorophyll a; b; a + b; and carotenoids) [Głuszek et al. 2008, Chitarra et al. 2016].

The application of mycorrhizal fungi in the form of a multi-component inoculum is highly promising and should be recommended in integrated tomato production systems as an element supporting natural plant physiology, immunity, and protection against phytopathogens. The effectiveness of commercial mycorrhizal fungal inoculants naturally depends on the quality and quantity of the inoculum, compatibility with cultivated plant species, native microbial communities, environmental factors, and soil management practices [Basirus et al. 2021]. The pot experiments confirmed the multidirectional impact of the mycorrhizal inoculum as a factor improving photosynthesis, contributing to increased root weight, and promoting a higher abundance of antagonistic fungi (*Trichoderma* spp.) in plant roots. The present findings indicate that plant mycorrhization can reduce the use of certain agrochemicals (mineral fertilizers and fungicides) and improve the safety of the harvested crops.

CONCLUSIONS

1. Plant mycorrhization reduced the total number of bacteria and fungi in the tomato rhizosphere.

2. The mycorrhizal inoculum increased the abundance of antagonistic fungi of the genus *Trichoderma* in tomato roots.

3. Plant mycorrhization increased the fresh and dry weight of tomato roots, although the increase was not statistically significant.

4. Leaves of mycorrhized tomato plants showed higher SPAD values and total chlorophyll content, although these differences were not statistically significant compared to control plants.

SOURCE OF FUNDING

This research was funded by the University of Life Sciences in Lublin, Poland (project SUBB.WOK.19.019).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

A.J.: working on a conception and assumptions. A.J., W.K.: developing the methods. A.J., W.K., E.P.: conducting research. A.J., W.K., E.P.: writing a manuscript, review and editing.

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