

## EFFECT OF MYCORRHIZA INOCULATION OF PEPPER SEEDLINGS (*Capsicum annuum* L.) ON THE GROWTH AND PROTECTION AGAINST *Fusarium oxysporum* INFECTION

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### ABSTRACT

A study was conducted to investigate the ability of arbuscular mycorrhizal fungi (AMF) in enhancing the growth and control of a root rot caused by different isolates of *Fusarium oxysporum* in sweet pepper seedlings. The plants were grown in plastic pots filled with sterilized horticultural soils. There were four treatments applied as follows: Fo (seedlings infected with fungus), M (seedlings inoculated with mycorrhizal fungi), Fo + M (seedlings inoculated with mycorrhiza and infected with fungus) and control. A randomized experiment was used and the growth, disease index, and photosynthetic activity of the plants were measured after 4 weeks. The investigations showed that the mycorrhizal inoculation had a protective effect on the *F. oxysporum*-infected pepper seedlings. The plants were characterized by a higher growth rate and a lower disease index than those growing only in the presence of the pathogenic fungus. The roots inoculated with mycorrhizal fungi were better developed than those infected only with *F. oxysporum*. The mycorrhiza contributed to an increase in the photosynthetic activity of the pepper seedlings.

**Key words:** mycorrhiza, *Fusarium oxysporum*, pepper seedlings, disease index, photosynthetic activity

Sweet pepper is a vegetable with great economic importance. It is highly appreciated for its high nutritional value and flavor. Pepper fruits are abundant in C, E, B-group vitamins, and carotenoids ( $\beta$ -carotene,  $\beta$ -cryptoxanthin). These compounds inhibit free radical formation in the human organism [Kaur and Kapoor 2001, Perucka and Materska 2007]. During their growth, pepper plants are exposed to a number of pathogenic fungi, which can reduce the nutritional value of the fruit, induce rotting during the storage period and even cause the production of harmful mycotoxins. The increase in the pepper cultivation

area in Poland promotes development of harmful organisms. One of the phytosanitary problems in pepper plantations are fusarioses caused by fungi from the genus *Fusarium*, in particular *F. oxysporum* f. sp. *lycopersici* [Pereira et al. 2016]. The fungus is widespread in nature and can infect plants throughout the vegetation season causing plant rot and wilting [Wagner et al. 2007, Jamiołkowska 2009, Rataj-Guranowska and Pukacka 2012].

The increased requirements imposed on consumed foods prompt application of alternative production methods. Formulations containing living

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organisms or other natural compounds are recommended for plant protection. Mycorrhizal inocula improving plant health are one of such products [Lixuan et al. 2010, Jamiołkowska et al. 2017]. Mycorrhizal fungi are widespread in the soil environment, where they play an important role. Mycorrhiza is a symbiotic association between fungi and roots of higher plants, which consists in providing plants with hardly available elements, thus exerting a favorable effect on the environment and crop plants. Mycorrhizal fungi improve water and nutrient uptake from the soil, which contributes to a better plant growth. The function of mycorrhiza is to protect plants against soil pathogens. They also increase the resistance of the root system to soil pathogens [Książniak et al. 2013, Jamiołkowska et al. 2018]. Useful soil microorganisms determine the mycorrhiza indirectly and directly and exert an antagonistic effect on pathogenic fungi. The use of mycorrhizal inoculations has mostly a positive impact on plant growth and health and is economically efficient due to the limitation of the use of plant protection products [Pereira et al. 2016, Jamiołkowska et al. 2017].

The aim of the study is to assess the effect of mycorrhizal inoculation on the growth, photosynthetic activity, and protection of sweet pepper seedlings (*Capsicum annuum* L.) against different isolates of *Fusarium oxysporum*.

## MATERIALS AND METHODS

**Plant material.** The experiment was carried out in the cultivation room at the University of Life Sciences in Lublin, Department of Plant Protection, in 2016. Seedlings of sweet pepper cv. ‘Roberta F<sub>1</sub>’ – Horticultural Seed and Nursery Company in Ożarów Mazowiecki, Poland) were used in the experiment.

**Mycorrhizal treatment.** A commercial mycorrhizal inoculum (Mycoflor, Końskowola, Poland) containing spores and dormant mycelium of endomycorrhizal fungi: *Rhizophagus aggregates* (syn. *Glomus aggregatum*), *R. intraradices* (syn. *G. intraradices*), *Claroideoglomus etunicatum* (syn. *G. etunicatum*), *Endogone mosseae* (syn. *G. mosseae*), *Funneliformis caledonium* (syn. *G. caledonium*), *Gigaspora margarita* were used for the experiment.

***Fusarium oxysporum* inoculum.** In the experiment, ten randomly selected *F. oxysporum* strains

were used as a group representing the diversity within the species. The *F. oxysporum* isolates (ACR21, AIG18, AEL14, AES14, AES25, ACR25, ACS22, AER8, ACS23, AES26) originated from own collection. Fungus isolates were obtained from tomato plants grown in the field in 2015. The fungal colonies used for plant infections originated from the 10-day spore cultures grown on glucose-potato agar in the thermostatic chamber at a temperature of 22°C.

Surface of sweet pepper seeds was disinfected with 0.1% sodium hypochlorite for 1 minute and then rinsed 3 times with distilled water [Jamiołkowska 2007]. Next, the seeds were germinated on plates filled with sterilized horticultural soil (peat moss) and quartz sand at a ratio of 2 : 1. When the first leaves appeared (after 7 days), the seedlings were planted into multiplates. 50 ml sterilized horticultural soil and 3 ml of mycorrhizal inoculum (M) were used for each seedling. The control seedlings were transplanted into sterile horticultural soil without the mycorrhizal inoculum (C). The seedlings were kept in a growth chamber at 22–23°C and 85% air humidity with a 14-h photoperiod and watered with sterile water as needed. After two weeks of growth, mycorrhized and non-mycorrhized pepper seedlings were transferred with overgrowth soil to pots (0.5 l, Ø 10 cm) filled with sterilized horticultural soil where *F. oxysporum* slices were placed and then covered with soil according to the method used by Jamiołkowska and co-authors [2011]. In the control combination, slices of medium without fungus were used. The following experimental combinations were used in the study: C – absolute control (seedlings growing without the mycorrhiza and without the fungus), M – relative control (seedlings inoculated with the mycorrhizal fungi), Fo – seedlings infected with the fungus, Fo + M – seedlings inoculated with the mycorrhizal fungi and infected with the fungus.

The experiment was carried out in a random block arrangement of 30 plants for each experimental combination (three plants in ten replicates). The pepper seedlings were grown in a growth chamber at 23–25°C and 85% air humidity with a 14-h photoperiod. All plants grew in the pots for 4 weeks. After this time, the size of plants and the number of healthy and diseased seedlings were recorded, and the photosynthetic activity of leaves was measured.

**Estimation of the disease index (DI) and percentage of diseased seedlings.** The plants were evaluated for the degree of infection after 4 weeks, using a 4-grade scale: 0 – no symptoms, 1 – small necrosis spots on all lateral roots, no symptoms on leaves or stem, 2 – necrosis of tap root and stem base, chlorotic leaves, 3 – completely rotten roots and stem base, death of plants. Then, the number of seedlings in each infection degree group was counted. The disease index was estimated for each replication using Townsend and Heuberger formula [Wenzel 1948]:

$$\text{Disease index} = (\sum a / \sum b) \times 100,$$

where a = sum of products of the numerical scale index (infection degree) and corresponding number of plants and b = total number of tested plants multiplied by the highest numerical scale index. Afterwards, the mean disease index was computed for each combination. The percentage of plants with disease symptoms was counted. Koch's postulate was met by re-isolation of the fungi from the diseased plants.

**Estimation of seedling growth.** The evaluation of the pepper seedlings growth was carried out by measuring the length of roots and aboveground parts of plants (cm). On this basis, the size of whole plants was determined.

**Estimation of photosynthetic activity of leaves.** Fluorescence parameters such as quantum yield of PSII photochemistry in the dark adapted state ( $F_v/F_m$ ), total quantum photosynthesis yield (Y), and the photochemical (qP) and non-photochemical quenching coefficient (qN), were measured with the PAM fluorometer (H. Waltz GmbH, Germany). Ten plants from each experimental combination and for each isolate were tested. For this test, one leaf from the same position on plants was selected.

**Statistical analysis.** Results from the obtained data were analyzed statistically using the SAS software. The significance of differences was evaluated on the basis of multiple Tukey's test at the significance level  $\alpha \leq 0.05$ .

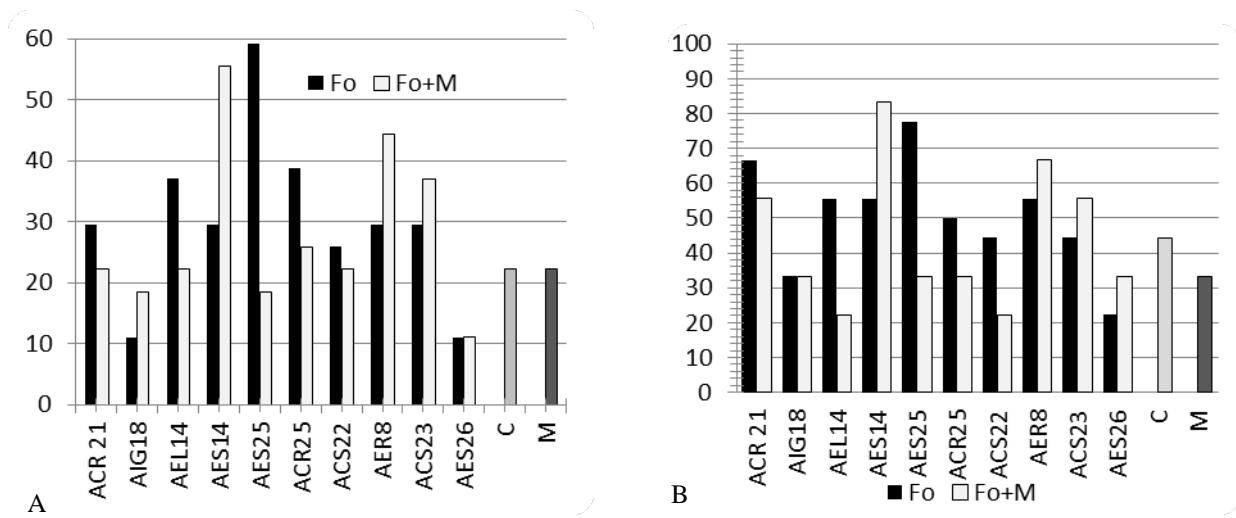
## RESULTS

### Disease index (DI) and percent of infected plants.

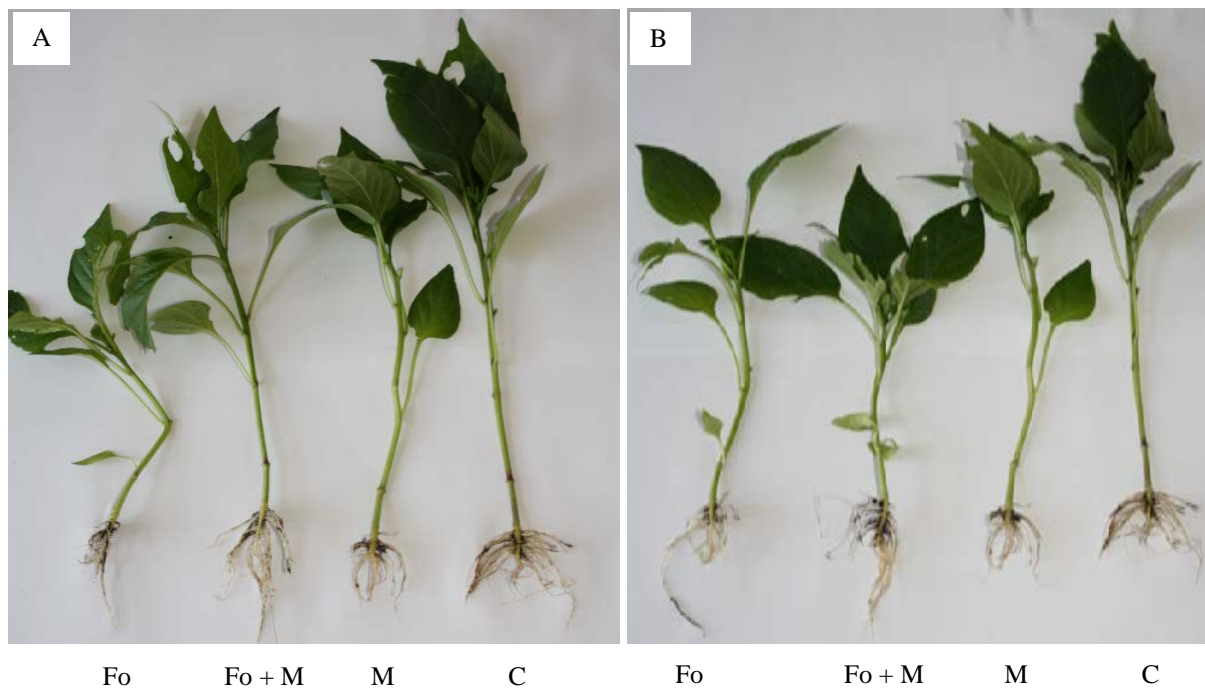
A varied degree of infection was observed in plants growing on the fungus-infected soil. The mean disease index in the *F. oxysporum*-infected plants (Fo) was

30.15%, which was higher than that in the control seedlings (22.2%) and in the mycorrhized and fungus-infected plants (Fo + M, 27.75%) (Fig. 1). The mycorrhization of the seedlings contributed to inhibition of the disease in the infected plants. A lower DI value was recorded for many mycorrhized and fungus-infected seedlings (Fo + M) in comparison with seedlings that were infected with the same isolates but without the mycorrhiza (Fo). The *F. oxysporum* isolates exhibited diverse pathogenicity towards the pepper seedlings. Isolates AES25, AEL14, ACR25, AES14, AER8, and ACS23 were found to have the highest pathogenicity, whereas AES26 and AIG18 exhibited the lowest pathogenicity (Fig. 1). In the mycorrhized and fungus-infected seedlings, the mycorrhiza contributed to a reduction of the seedling disease index in the range from 3.7% (ACS22) to 40.7% (AES25) in comparison with the infected but non-mycorrhized plants (Fig. 1). Seedlings infected with isolates AES14, AER8, and ACS23 were an exception; in this group, despite the prior mycorrhization treatment, the index of *F. oxysporum* infection was higher in the mycorrhized seedlings (Fo + M) than in the non-mycorrhized and fungus-infected plants (Fo) (Fig. 1). The percentage of seedlings with disease symptoms was different and depended on the fungal isolate. For isolates ACR21, AEL14, AES25, ACR25 and ACS22, there was a positive effect of the mycorrhiza and the percentage of plants with disease symptoms was lower than in the control (Fig. 1).

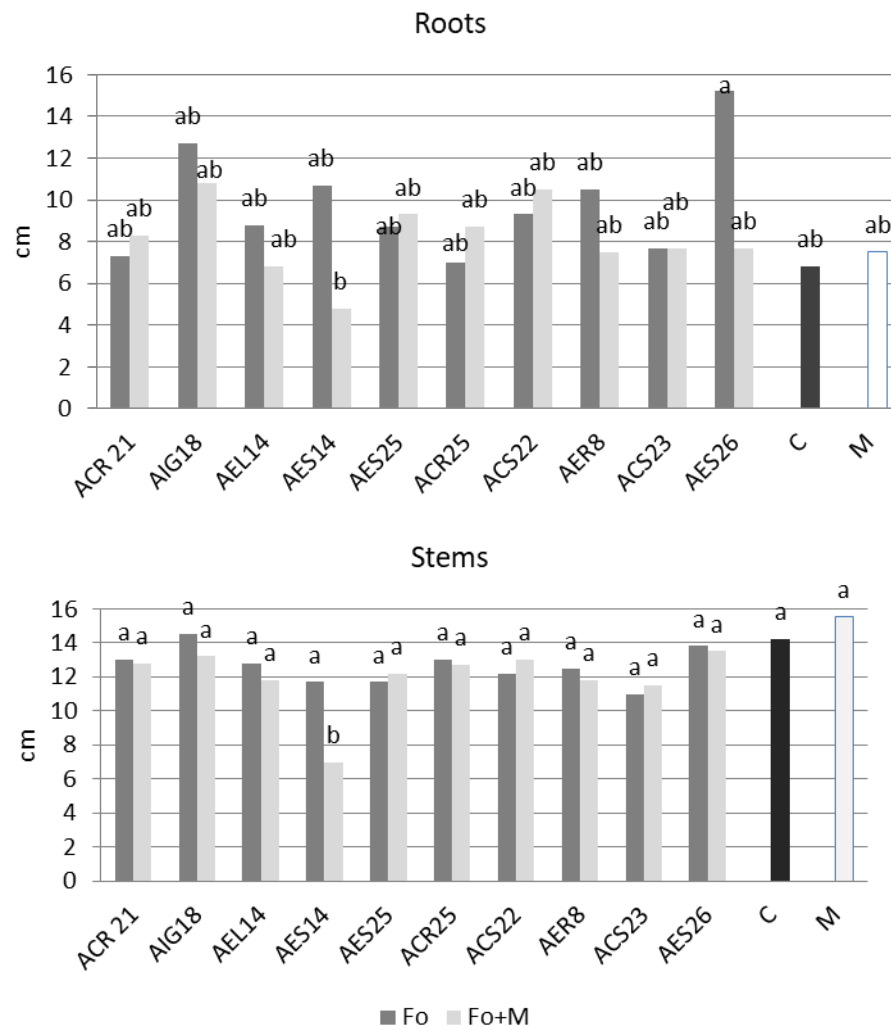
**Size of roots and stems.** The macroscopic analysis of plants revealed that the absolute-control seedlings (C) have well-developed root system with numerous lateral roots (mean length 6.8 cm) (Figs. 2–3). The relative-control seedlings (M) produced a more weakly developed root, which was however longer than that in the absolute-control plants (mean length 7.5 cm), but not significant. The fungus-infected seedlings (Fo) had poorly developed roots with clear necrosis symptoms (7.0–15.2 cm). The exception were seedlings infected with AES26 isolate, the roots of which were very elongated and statistically different from other experimental combinations. In turn, the lateral roots in the mycorrhizal fungi and *F. oxysporum*-infected seedlings (Fo + M) were strongly elongated and the tap root exhibited only few necrotic lesions (4.8–10.8 cm), but no statistical differences were observed between seedlings infected with other isolates (exception AES14 isolate) (Figs. 2–3).



**Fig. 1.** Disease index (A) and percentage of diseased pepper seedlings (B); C – absolute control, M – relative control, Fo – seedlings infected with the fungus, Fo + M – seedlings inoculated with the mycorrhizal fungi and infected with the fungus



**Fig. 2.** Pepper seedlings grown with the fungus, mycorrhiza and in the control groups; A – plant inoculated with isolate AER8; B – plant inoculated with isolate ACR21; note as in Figure 1

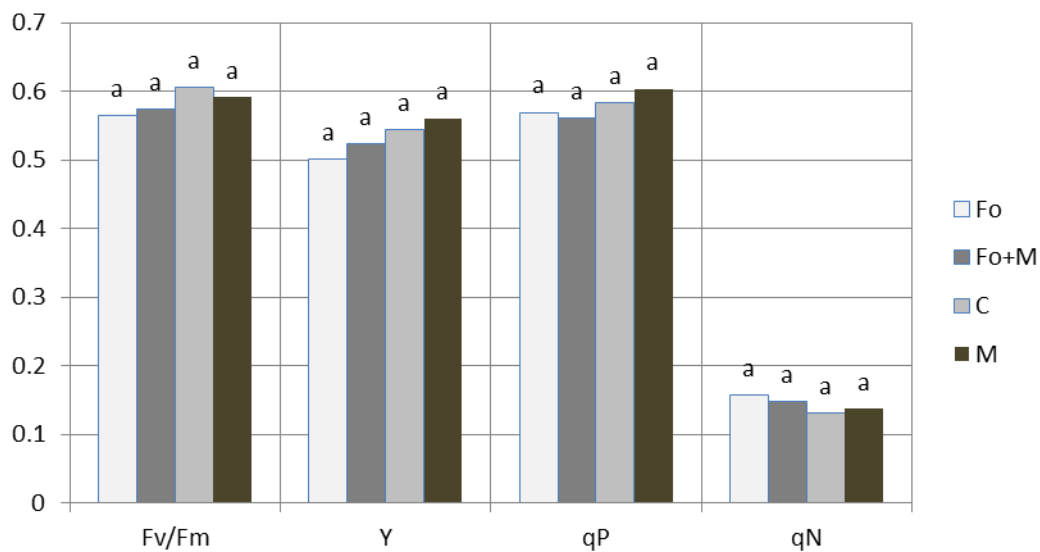


**Fig. 3.** Size of pepper roots and stems of seedlings inoculated with *F. oxysporum* (Fo), seedlings with mycorrhiza and *F. oxysporum* (Fo + M); values marked with the same letters (a, b, ...) for the size of plants do not differ significantly at 5% error (Tukey's test); note as in Figure 1

The stems of the *F. oxysporum*-infected plants (Fo) were longer (11.0–14.5 cm) than in the mycorrhized and fungus-infected seedlings (Fo + M, 7.0 – 13.5 cm), but shorter than the stems of the control plants (C, 14.2 cm; C + M, 15.5 cm) (Figs. 2–3). However, there was no significant effect of mycorrhizal fungi on the length of the stem (exception AES14 isolate) (Fig. 3).

**Photosynthetic activity of pepper seedlings in tested experimental combinations.** The analysis facilitated determination of the photosynthetic activity of pepper seedlings in different growth condi-

tions. Figure 4 shows the average photosynthetic activity of pepper seedlings for the tested experimental combinations. There was a beneficial effect of mycorrhization of seedlings on the plant photosynthetic activity. The mean values of maximal photochemical efficiency of PSII ( $F_v/F_m$ ) and total quantum photosynthesis yield (Y) in the case of the mycorrhized and fungus-infected seedlings were slightly higher than values noted in the seedlings infected with the pathogen and lower than in the control. However, the differences were not statistically significant (Fig. 4). The value of photochemical



**Fig. 4.** Photosynthetic activity of pepper seedlings in the experimental combinations; n – 10 samples; values marked with the same letters (a, b, ...) for the size of plants do not significantly differ at 5% error (Tukey's test);  $F_v/F_m$  – maximal photochemical efficiency of PSII, Y – total quantum photosynthesis yield, qP – photochemical quenching coefficient, qN – non-photochemical quenching coefficient; note as in Figure 1

cal quenching coefficient (qP) and the non-photochemical quenching coefficient (qN) for the fungus-infected plants was slightly higher than that for the control plants, which indicated faster photosynthesis quenching in the presence of the pathogenic agent (Fig. 3). Recorded differences were not statistically significant. The presence of mycorrhizal fungi contributed to the improvement in the photosynthetic activity of the mycorrhized seedlings despite the presence of the pathogen. The maximal photochemical efficiency of PSII ( $F_v/F_m$ ) was correlated with the plant disease index (DI). The increase in the disease index in the fungus-infected seedlings led to a decline in the maximum photosynthetic efficiency (dispersion of results along the trend line for the experimental combinations). In turn, despite the increase in the disease index in the mycorrhized and fungus-infected seedlings, there was an increase in the maximum efficiency of the PSII photosynthetic system (Figs. 1, 4).

## DISCUSSION

The present investigations indicate that the mycorrhizal inoculum had a protective effect on the

*F. oxysporum* fungus-infected pepper seedlings. The plants were characterized by higher growth rates and a lower disease index than those growing only in the presence of the pathogenic fungus. Mycorrhizal inocula contribute to the elimination of pathogenic microorganisms such as *Verticillium* spp., *Fusarium* spp., and *Phytophthora* spp. from the soil environment, simultaneously contributing to increased plant resistance [Perrin 1990]. Głuszek and co-authors [2008] reported a decline in pathogen populations and reduced severity of plant infection in the presence of arbuscular mycorrhizal fungi (AMF). The symbiosis of plants with AMF enhances plant defense reactions, which may trigger mechanisms of early response to the attack of soil pathogens [Hao et al. 2005]. McAllister and co-authors [1997] found that lettuce and maize inoculated with an endomycorrhizal fungus exhibited increased resistance to the negative effects of *Fusarium oxysporum* infection. Similar investigations were carried out on cucumber seedlings to assess the effect of inoculation with a mycorrhizal fungus on plant resistance to *F. oxysporum* infection [Hao et al. 2005]. Different results were obtained by Al-Hmoud and Al-Momany

[2015], who demonstrated no significant differences between *Fusarium* spp. – inoculated tomato plants and a mycorrhized control. Nevertheless, it was found that the percentage of infected plants was by 8% higher than in the mycorrhized plants.

The use of mixed mycorrhizal inocula is more effective and yields better results than the use of one mycorrhizal fungus species. Selection of the best native mycorrhizal fungus species offers appropriate protection not only for plants but also for soils and the environment colonized by pathogenic agents [Zeng 2006]. Own studies did not show any significant influence of mycorrhizal fungi on the growth of roots and stems of mycorrhizal seedlings infected with fungus. However, the mycorrhized and *F. oxysporum*-infected seedlings were characterized by a higher growth rate than the fungus-infected seedlings. The macroscopic analysis revealed also that the mycorrhized plants had a better developed root system and displayed no necrosis symptoms, in comparison with the roots of the *F. oxysporum*-infected seedlings. The roots of the fungus-infected plants were strongly reduced but, at the same time, they were longer, which indicates that the plant penetrates deeper layers of the substrate to absorb nutrients outside the fungal inoculum region. The finer and shorter roots of the relative-control plants, compared with those of the absolute-control seedlings, indicate that the root system is reduced and the root hair function is taken over by the hyphae of arbuscular mycorrhizal fungi (AMF). As reported in the literature, establishment of plant-mycorrhizal symbiosis modifies the plant growth and root architecture. The root system is more highly branched and produces a larger number of lateral roots. Consequently, the better and more efficient soil penetration contributes to better water and nutrient uptake. Mycorrhizal fungus hyphae colonizing the roots form a mechanical barrier against pathogens, which directly protects the root system from soil-borne diseases [Mwangi et al. 2011, Jamiołkowska et al. 2017]. Investigations conducted by Al-Hmoud and Al-Momany [2015] demonstrated significant differences in the height of mycorrhized and non-mycorrhized plants inoculated with *Fusarium oxysporum*. Contrasting results were presented by Książniak and co-authors [2013], who found that inoculation of rhododendron plants with mycorrhizal fungi had a neutral effect on the growth of these plants. A low impact of mycorrhization on

the growth and flowering of geranium plants was reported by Nowak [2007]. Still, most research results have evidenced the beneficial effect of mycorrhizal inoculation on the growth of pepper seedlings.

Mycorrhiza has an effect on a number of physiological processes in plants, e.g. photosynthesis. Any reduction in the intensity of this process is associated with a decline in a yield quantity and quality. An important indicator of the effect of stress factors on plants is measurement of chlorophyll fluorescence, which exhibits changes in the PSII photosystem activity, i.e. it determines the normal course of the light phase of photosynthesis [Sawicka and Michałek 2005]. In the present experiment, the use of mycorrhizal inoculum resulted in an increase in the quantum yield of the PSII photosystem and the total quantum yield of photosynthesis. However, there was an increase in the non-photochemical fluorescence quenching, which indicates disturbances in photosynthesis and rapid quenching of this process. Mycorrhized plants can compensate for the higher carbohydrate demand by increasing the photosynthesis rate. It was shown that an increased size of a mycorrhized root system in white clover was the cause of enhanced photosynthesis [Nowak and Nowak 2013]. Many authors have reported that a decline in the  $F_v/F_m$  value indicates damage to photo-inhibitors when plants are exposed to environmental stresses [Bagheri et al. 2011]. Other studies have demonstrated that mycorrhization caused an increase in the intensity of photosynthesis in geranium plants, which was associated with an increase in the ratio of variable to maximum chlorophyll fluorescence [Nowak and Nowak 2013]. Zhu [2012] reported that maize plants inoculated with mycorrhiza were characterized by an increase in photosynthetic indices in comparison with non-mycorrhized plants. The results indicate that stress in plants destroys the structure and function of the PSII reaction center and disrupts the electron transport in the photosynthetic apparatus, whereas plant symbiosis with AMF mitigates some adverse side effects. As reported by Michałek [2004], the decrease in the value of chlorophyll fluorescence induction parameters indicates lower efficiency of primary photosynthesis in the PSII photosystem. Present results demonstrate that the effect of mycorrhizal inoculum on chlorophyll fluorescence in pepper leaves can be one of the indicators of their tolerance to adverse environmental conditions.

## CONCLUSIONS

Mycorrhization of pepper seedlings contributed to the inhibition of the disease, because the disease index (DI) of mycorrhized seedlings infected with *F. oxysporum* was lower than for seedlings infected with fungus. Mycorrhization of pepper seedlings did not significantly affect the length of roots and stems, but mycorrhized and infected *F. oxysporum* seedlings had more strongly developed root system with less necrosis than seedlings infected only with fungus. Studies have shown an increase in fluorescence parameters such as quantum yield of PSII photochemistry in the dark adapted state (Fv/Fm), total quantum photosynthesis yield (Y), and the photochemical quenching coefficient (qP) for seedlings treated with mycorrhizal fungi and infected with *F. oxysporum* and faster quenching of photosynthesis in mycorrhized seedlings than in seedlings infected with fungus.

## ACKNOWLEDGEMENTS

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