APPLICATION OF MYCORRHIZAL FUNGI IN THE PRODUCTION OF TOMATOES IN A CLOSED SPACE

ORIGINAL SCIENTIFIC ARTICLE

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ABSTRACT:
Agricultural production requires alternative solutions for the application of pesticides and mineral fertilizers. One, reliable solution is definitely the application of mycorrhiza, because in addition to reducing the use of pesticides and mineral fertilizers, it increases the resistance of plants to biotic and abiotic stresses. Mycorrhizal inoculation improves nutrient and water uptake by many host plants. Arbuscular mycorrhizal fungi (AMF) grow in close association with plant roots and play an important symbiotic role in the uptake and transfer of water and nutrients in the root system; in exchange, the plant supplies the fungus with sugars. The aim of this research is the possibility of controlled application of mycorrhizae and examination of the influence of the symbiosis of mycorrhizae and tomato plants on achieving better yields, resistance to diseases and stress factors, as well as on plant growth itself. The main hypothesis of this field study was that the symbiosis of an arbuscular mycorrhizal fungus with tomato roots would increase crop growth and yield under stressful abiotic conditions. The test was conducted in a 300 m^2 greenhouse on an agricultural estate in Ustikolina with the application of mycorrhiza in the amount of 1g, 3g and 6 g., for each plant. The achieved results gave a clearer picture of the importance of the symbiotic action of mycorrhizal fungi and tomatoes in the form of improved growth and fruiting.

KEYWORDS: mycorrhization, symbiotic relationship, yield, stresses

INTRODUCTION

In agricultural production, the application of beneficial microorganisms, such as arbuscular mycorrhizal (AMF) fungi, reduces the use of pesticides, artificial fertilizers and increases the tolerance of plants to abiotic factors. About 96.5% of the global rural area is under the influence of abiotic stresses [1]. Drought can cause a yield loss of 13% to 94% under several conditions depending on the intensity and duration of the drought [2]. World agriculture is in the process of transition to sustainable production, and this again requires the reduction of chemical inputs and the preservation of the richness of the microbiome and biodiversity. Plants should be viewed through their association with microorganisms and positive effects on the condition and health of the plants themselves [3]. In nature, there are communities between plants and microorganisms that can be beneficial or harmful to the host plants. One of the ways towards ecologically acceptable and sustainable crop production is the establishment of a beneficial interaction between plants and microbes [4]. Mitigation of damage from stress can be achieved with a symbiotic mixture of mycorrhizal fungi. Studies have shown that when the plant was faced with multiple abiotic stress factors (lack of nutrients and high concentrations of various heavy metals), co-inoculation of fungi belonging to different families was more effective than mono-inoculation in improving biomass, mineral nutrition, Ca/Mg ratio and heavy metal tolerance of plants in soil [5]. Mycorrhizal symbiosis is formed by fungi with plant roots due to a mutually beneficial relationship. It is one of the first examples of mutual relationship on Earth. In addition to providing plants with nutrients and water, mycorrhizae provide additional benefits in combating biotic and abiotic stresses [6]. The fungus develops hyphae that take up carbohydrates produced by the host through photosynthesis, the fungi help the plant take up water and immobile soil nutrients such as phosphorus, copper and zinc. In most natural environments, symbiosis is a normal occurrence. In terrestrial environments, approximately 85-90% of plant species form a symbiosis with soil fungi (symbiosis defined as mycorrhiza) [7]. The use of mycorrhizal fungi has a positive effect on the concentration of potassium and calcium in the fruit and a better water potential [8]. in vegetable production, the use of mycorrhizal fungi is desirable, even necessary, because it can have a positive effect on yield and quality even though smaller doses of mineral fertilizers are applied. Inoculation of the root system with mycorrhizal fungi, sweet pepper, allows to reduce the amount of mineral fertilizers without significantly reducing the yield and quality of fruits [9].

The use of mineral fertilizers has theoretically reached its maximum use and there will be no increase in yield due to the use of fertilizers. Poor fertilizer management poses a threat to the environment. In order to avoid negative consequences for the environment, the efficiency of fertilizers must be significantly increased [10]. Increasing the yield by applying a larger amount of chemical fertilizers to the soil is a danger to the health of people and the environment, so that the soil and plants cannot maintain healthy production for a long time. Reducing the impact on the negative effects of chemicals on human health and the environment are the main reasons for increasing restrictions on the use of chemicals and increasing biocontrol [11,12]. Beneficial microorganisms play an important role in high-quality agricultural production, and reducing the use of chemical inputs also helps prevent yield reductions. Although bacteria and fungi have been repeatedly demonstrated over the past 150 years to promote plant growth and suppress plant pathogens, this knowledge has not been widely used in agricultural biotechnology. Despite extensive research on biocontrol and the potential of BCA use as an alternative to chemicals, global reliance on BCA use remains relatively insignificant [13].

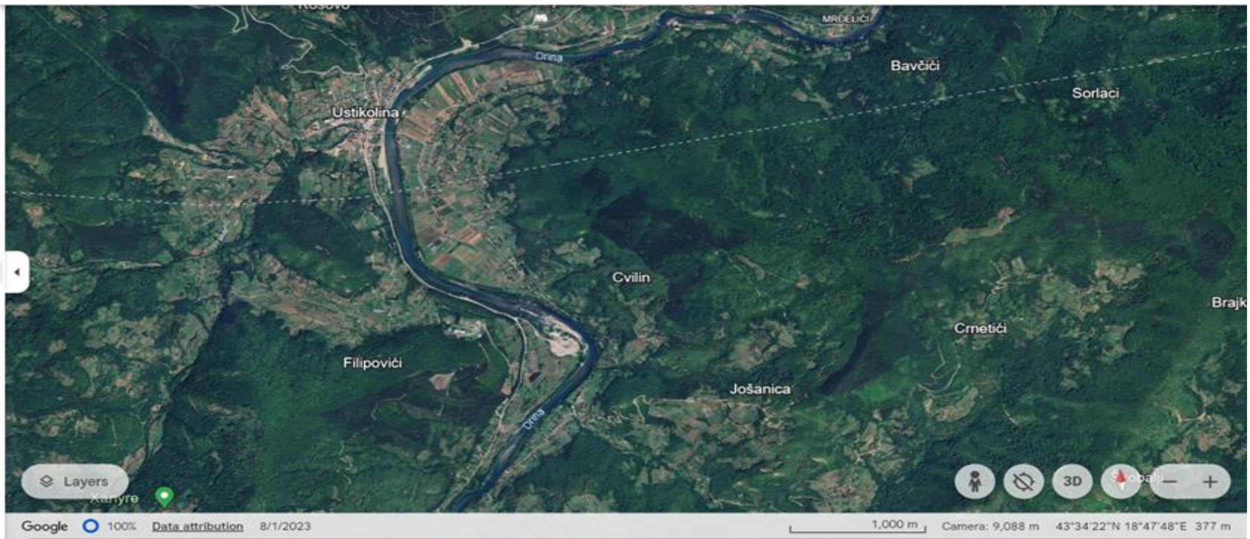
Mycorrhizal fungi that were applied to the root system during seedling production had a positive effect on the yield and biometric characteristics of peppers, with the fruits of the thickest pericarp and the largest mass. Vegetables (*Solanaceae and Cucurbitaceae*) are often under the influence of abiotic factors, which have changed with climate change, and pressure is exerted on the plants to survive and to give good yields [14]. Mycorrhizae improve plant tolerance to several abiotic stresses by various physiological, functional and biochemical changes in plants.

As a result of research on the effects of mycorrhizal inoculation with two species (*Glomus clarum* and *Glomus caledonium*) and three different inoculation treatments (sowing, transplanting and sowing + transplanting) applied to peppers grown hydroponically on a perlite substrate, the effect of *G. clarum* and *G. Caledonium* increased the yield by 29% and 21%, respectively, compared to control plants [15]. On mycorrhized tomato plants, shoot, root and total seedling dry biomass increased significantly with AMF (Arbuscular mycorrhizal fungi) inoculation compared to the control at 14, 28 and 42 days of measurement after inoculation [16]. Arbuscular mycorrhizal fungi (AMF) are widespread soil fungi that form associations with the roots of most (>80%) terrestrial plant species [17].

Tomato plants grown in substrates receiving 50% mineral fertilizers showed the highest level of mycorrhization, showing a frequency (F) of 100% and an intensity (M) of 63%. Importantly, the combination of inoculation with a reduced dose of NPK fertilizer (50% of the recommended amount) resulted in significantly increased concentrations of calcium, potassium, iron, zinc, and phosphorus in plants, which can be attributed to enhanced microbiome root colonization [18]. Numerous previous studies have confirmed the effective use of AMF inoculum either alone or in combination with NPK fertilizers or different levels of phosphorus to improve plant growth, improve mineral nutrient uptake and increase crop yield [19,20].

MATERIALS AND METHODS

The survey was carried out on an agricultural estate in the Municipality of Foča in FBiH, the place of Ustikolina. The place is located in the valley of the river Drina, surrounded by mountains, with a rich and fertile plain, Cvilinsko polje with geographical coordinates: 43°34'22"N; 18°47'48"E.



Picture 1. Display of test location- Ustikolina.
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Sandy soil prevails in this locality, formed as alluvium of the old course of the Drina river. The analysis of the physical and chemical characteristics of the soil, i.e. the analysis of the soil, was done at the Faculty of Agriculture and Food of the University of Sarajevo. The analysis of the chemical properties of the soil indicates that the soil has a neutral to slightly alkaline reaction, medium humus content, but very rich in available potassium and very poor in available phosphorus (table 1).

Table 1. Results of physical and chemical characteristics of soil

Characterictics	Measure unit	Result
pH in H2O	-	7,31
pH in KCl	-	7,05
K2O	mg/100 g	108
P2O5	mg/100 g	< 1,0
Humus	-	2,22%
Carbonates	-	Soil without carbonates

After the own production of seedlings, the planting of seedlings was started. The seedlings were planted in two rows with a spacing of 40 x 50 cm and on three beds. The distance between the beds is 60 cm. During planting, mycorrhiza was applied, in the amount of 1g, 3g and 6 g. for each plant. In the test, 20 plants were used for each variant of mycorrhiza, 20 control plants. At the time of planting, tomato seedlings had an average height of 18 cm with 4 to 5 developed leaves and were about 60 days old.

The test was conducted in a greenhouse 5 meters wide and 60 meters long (300m2), three beds were formed with a planter inside the greenhouse. The space between the rows is mulched with straw. Tomatoes of the Marathon F1 variety from the Superior company, in the apple type, were used in the test. The mycorrhizal agent under the trade name Mycoriza-Rhizo-vam Basic contained the spores of *Glomus intraradices* 10^6 cells/g.

Picture 2. Micoriza- Rhizo-vam Basic

Morphometric measurements included; growth height measurements, average and total yield.

Measurements were made on each of 20 plants, 3 treatments of 20 plants and 20 control plants by measuring the plants with a meter to determine their growth. The yield was measured from each plant (on a digital scale) and then the average and total yield was determined.

Monitoring the presence of diseases on tomatoes was carried out by frequent visual inspection. Frequent visual inspection of tomato plants allows for rapid recognition of disease symptoms, such as changes in leaves, stems or fruits.

Early recognition of fungal plant diseases is crucial for timely treatment, which can prevent significant losses in agriculture. Molecular analysis offers high accuracy, is often expensive and time-consuming[21].

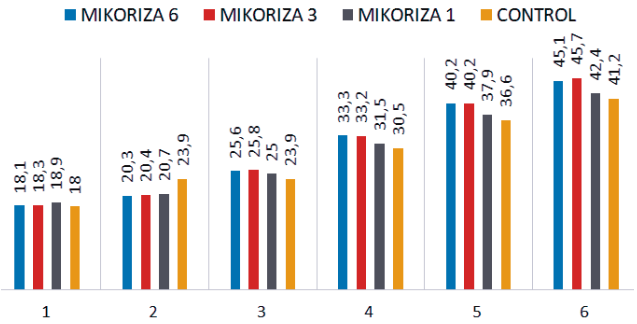
Good and experienced experts are required to diagnose plant diseases with the naked eye. This method can be accompanied by significant error [22].

RESULTS AND DISCUSSION

Three concentrations of mycorrhizal agent 1g, 3g 6g and a control group of plants were used in the work.

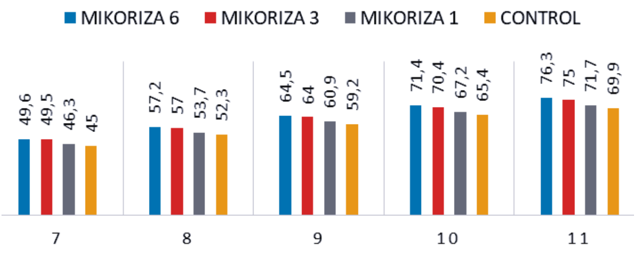
In the first two weeks of growth, from planting and applying the product, no difference in growth was noticed and the plants looked quite uniform.

The difference in height starts to be noticed already in the third week in which the plants from the "mycorrhiza 6 g" and "mycorrhiza 3 g" groups show a better growth tendency compared to the plants from the "mycorrhiza 1 g" and "control" groups, which can be seen from graph 1.



Graph 1. Tomato groups growth in first six weeks

At the end of the sixth week, the plants from the "mycorrhiza 6 g" and "mycorrhiza 3 g" groups had better growth, where the difference in plant height was 3 to 4 cm compared to the other two groups.



Graph 2. Tomato groups growth in period from 7 to 11 weeks

Towards the end of the 11th week, a significant difference in the growth of the tested groups can be observed, where the plants from the "mycorrhiza 6 g" group showed better adaptability, which resulted in an average plant height of 26.7 cm compared to the plants from the control group, where the growth was an average of 24.9 cm, i.e. the difference between these two groups is 1.8 cm, which is proven by Graph 2.

The standard deviation during this period ranged from 9.98 cm (control) to 10.72 cm (MYCORHIZ 6), indicating uniform growth within each group, without drastic oscillations between plants.

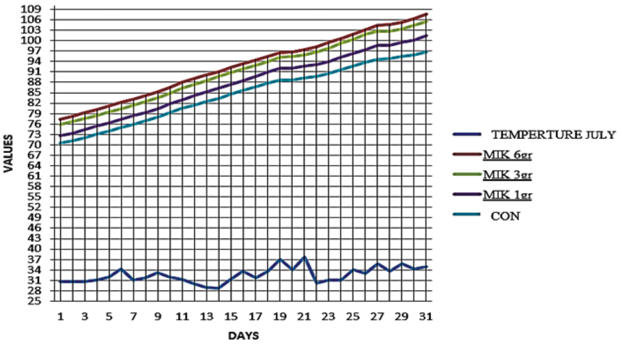
In order to determine the statistical significance of the difference between the treated groups and the control, an independent samples t-test was applied. The results showed that none of the differences between the treatments and the control were statistically significant (all $p > 0.05$), although a tendency for better growth was observed in mycorrhizal plants.

In the July, the first higher air temperatures were recorded, and in order to reduce the stress factor for the plants, foliar biostimulation and one fertigation supplement were performed.

The symbiosis between AMF and plants plays a key role in alleviating the stressful impact of drought, as it enables better availability of water and nutrients, reduces water loss through transpiration and increases plant resistance to stressful conditions. According to literature data, AMF inoculation increased the rate of photosynthesis and transpiration by 40% to 70%, doubled the leaf area and increased the relative water content in the leaves of the plant by 25%. By studying the effect of mycorrhizal inoculation on the total yield, a 23% increase was found in inoculated plants (57.1 t ha) compared to non-inoculated (43.93 t ha) plants. Quantitative yield estimates of fruit number escalated to 35 numbers in inoculated plants (30.6 fruit plants) instead of non-inoculated (19.9 fruit plants) [23].

The application of commercial AMF inoculum (*F. mosseae* and *Septoglomus constrictum*) caused an increase in the total height of tomato plants despite chemical fertilization in inoculated plants (48.4 cm plant -1) compared to non-inoculated plants (39.7 cm plant -1) with an increase of 18% [24].

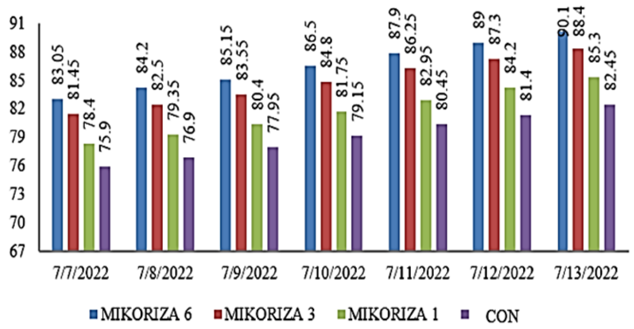
From Graph 3, in which the maximum temperature peak of around 37ºC was recorded, we can see that the plants treated with mycorrhizal fungi more easily tolerated the temperature stress, where they maintained their tendency to grow.



Graph 3. Growth stability in relation to the maximum recorded temperature

At the end of the first week of July, foliar top dressing was carried out with the preparation Slavol, which is a liquid microbiological fertilizer and growth stimulator, certified for use in organic and traditional agricultural production in a concentration of 1.5% according to the manufacturer's recommendation and through an irrigation system with nettle decoction 0.01%.

Urtica dioica is considered one of the best plant liquid nitrogen fertilizers that promotes the formation of chlorophyll. It grows very quickly and absorbs minerals and other phytonutrients, so it is considered a bioaccumulator. In addition to the already mentioned elements such as nitrogen, sulfur and boron, it is rich in iron, silicon, calcium, potassium and phosphorus, which are generally substances that plants need for growth. Foliar nutrition and fertigation were carried out in the morning hours, during which the response of plant stimulation to growth caused by the above-mentioned two agents was monitored for seven days, supported by mycorrhizal fungi, which can be seen in graph 5. The plants from the "mycorrhiza 6 g" group showed the greatest increase at the end of the examined week, where the total weekly increase was 7.05 cm, and showed a better response to foliar nutrition compared to the control group, where the weekly increase was 6.55 cm.



Graph 4. Plant growth (from 7th to 13th day) after fertirigation

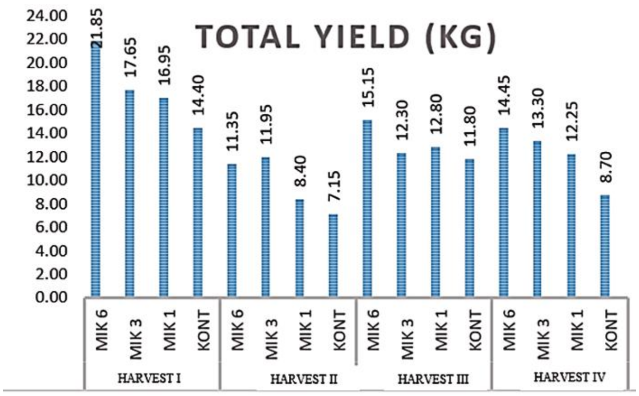
The plants from the groups "mycorrhiza 3 g" and "mycorrhiza 1 g" had a smaller increase caused by foliar feeding compared to the group "mycorrhiza 6 g" by 0.1 cm and 0.15 cm, which is negligible considering the difference. Comparing these two groups with the control, the difference in growth increases by 0.4 and 0.35 cm in favor of mycorrhizae.

In addition to growth, the influence of mycorrhizae on the quality of fruiting was investigated. Four harvests were carried out, during which the values of the total yield of the examined group, the average yield of an individual plant and the average weight of the fruit were monitored.

The total yield of individual groups varied widely, at the first harvest the highest yield was achieved by plants from mycorrhiza 6g with an amount of 21.85 kg, which we can see from graph 6. Compared to mycorrhiza 6g, mycorrhiza 3g and mycorrhiza 1g, achieved lower yields by 4.2 kg and 4.9 kg, but had a higher yield compared to the control by 3.25 kg and 2.55 kg.

In the first harvest, the mycorrhizae showed their potential and produced an average of 0.5 to 1.5 kg more fruits compared to the control group.

During the second harvest, the total yields fell due to high temperatures during the day, and the yields of mycorrhiza 6g ranged around 11 kg, and a slightly higher yield was achieved by the mycorrhiza 3g group with an average of 11.95 kg, which is 4.8 kg more than the control plants.



Graph 5. Total yield of examined group of plants

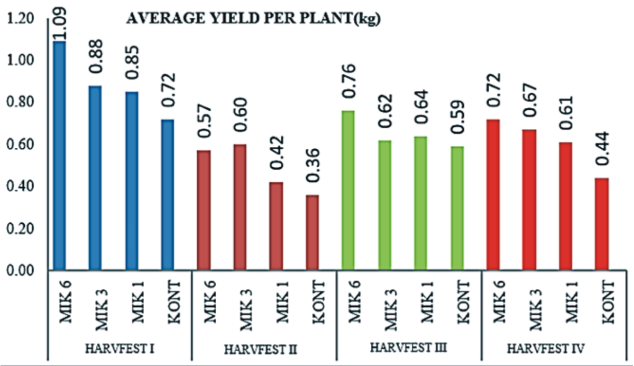
Due to the partial improvement of temperature conditions in the greenhouse and stimulation with foliar nutrition, the yields of all mycorrhizae increased slightly in the third harvest. Plants inoculated with mycorrhiza in the amount of 6g produced an average of 15.15 kg of fruits in the third harvest, and comparing them with the control group, they achieved a higher yield by 3.35 kg.

Analysis of the total yield, standard deviation, shows that MIK 6 generally gives a higher yield than the others, but the differences are not statistically significant.

In addition to the total yield, in order to get a clearer picture of the benefits of mycorrhiza application through the yield, the average yield per plant and the average weight of the fruit were considered.

The average yield per plant varied during the entire growing season through four harvests, so in the first harvest, according to Graph 6, the highest yield per plant was achieved by mycorrhiza 6g with an average of 1.09 kg and an average fruit weight of 300 grams. Compared to the control during the same harvest, it is a difference of 0.37 kg per plant and 70 grams per fruit, converted into fruits, it is 1.48 fruits with an average weight of 250 grams.

According to the standard deviation, MIK 6 generally gives a higher yield than the others, but with visible variability.



Graph 6. Average yield per plant

In the second harvest, yields fell due to unfavorable temperature conditions prevailing in the protected area despite the shade net, where we achieved the highest average yield per plant in mycorrhiza 3g, with the highest average fruit weight of 0.20 kg for that harvest.

By stabilizing the temperature, the yields improved significantly in the third and last harvest, where in the last harvest a stabilization was recorded in terms of the average fruit weight, where the fruits in the mycorrhizal group were almost uniform.

CONCLUSION

Mycorrhizae help tomatoes better withstand stressful conditions, such as drought and high temperatures.

The difference in the height of the plants was 3 to 4 cm. After foliar feeding and irrigation, after heat stress, plants inoculated with 6g each had an average growth of 7.05 cm in one week compared to the average growth of control non-inoculated plants of 6.55 cm.

The use of mycorrhiza resulted in higher tomato yields. In the first harvest, the inoculated plants showed their potential and produced an average of 0.5 to 1.5 kg more fruits compared to the control group. In the second harvest, the total yields are higher by 4.8 kg, in the third harvest by 3.35 kg and in the last fourth harvest by 5.2 kg.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

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