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The Influence of Mycorrhizal Fungi on the Growth of Apple and Sour Cherry Maidens Fertilized with Different Bioproducts in the Organic Nursery

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Abstract: Apple/*Malus x domestica* cv. 'Topaz'/M26 and sour cherry cv. 'Debreceni Bötermö'/*Prunus mahaleb* L. maidens were fertilized with different organic fertilizers and soil amendments in an organic nursery. A mycorrhizal inoculant, Micosat, was added to the non-fertilized control and to combinations fertilized with various bioproducts. Under assessment was the effect of nutrient delivery on the growth and development of maiden fruit trees focussing on tree height, thickness of the tree trunk, the number and the length of lateral shoots in each treatment combination. It was found that the introduction of mycorrhizal fungi into the soil without fertilizer application significantly improved the growth and development of apple and sour cherry maidens. Mineral fertilizer application (NPK) combined with mycorrhizal inoculation had a negligible effect on the above features. In the presence of bioproducts such as granular manure, Humus Active + Aktywit PM, BF Amin, Vinassa and Florovit Eko, the mycorrhizal fungi were more biologically active than in the absence of these products.

Key words: Biopreparation, fertilization, maiden fruit tree, mycorrhiza, ecological nursery.

1. Introduction

Lack of detailed information on the impact of traditional organic fertilization with manure and very few data on new organic fertilizers, plant extracts, and bacterial and mycorrhizal inocula are real obstacles to the development of innovative technologies of cultivation and fertilization of plants in the agricultural sector [1, 2].

Mycorrhizal fungi live in symbiosis with the majority of plants growing in the wild as well as with cultivated crops. The symbiosis generally results in an increase in the uptake of minerals (P, N, Mg, K, and microelements) by plants and higher crop yields, promotes vigorous growth of plants and makes them more resistant to environmental stresses, such as water deficit or excessive overheating of the substrate [3, 4]. There are, however, examples of reverse action, in which some species of plants eliminate or inhibit the biological activity of mycorrhizal fungi. To a large extent, this effect often depends on the type of agrotechnical treatments, plant species and cultivar, soil fertility and degree of acidification, planting density, soil temperature, and the cultivation system [2, 5].

Application of mycorrhizal inocula can increase species diversity of mycorrhizal fungi in the rhizosphere and consequently improve the growth, yield and yield quality of cultivated fruit crops [6, 7]. Determination of the optimal conditions for the coexistence of a plant and symbiotic fungi is key to obtaining positive effects of mycorrhization [5].

Most of the attention has been focused on the influence of mycorrhiza on the growth and fruiting of strawberry plants [2, 8-10].

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New products for improving soil fertility and technologies being introduced into organic production are studied in detail also in terms of their effect on the behaviour of soil microorganisms [2]. Numerous forms of organic fertilizers and soil conditioners have been evaluated by many authors, both on herbaceous and woody plants [11-14]. Soil enrichment with organic bioproducts containing beneficial microorganisms, including bacteria and mycorrhizal fungi, provides a real opportunity for improving the growth and fruiting of plants [1, 15-16]. The evidence for this are recent studies of fruit trees grown by organic methods in nurseries [13, 17-19].

The aim of the present study was to assess the biological effects of mycorrhizal fungi used in an organic nursery of fruit trees in combination with various bioproducts containing nutrients and active substances (i.e. plant hormone-like substances, enzymes) to more intensively stimulate the growth and development of maiden fruit trees.

2. Materials and Methods

The study was conducted in 2011-2013 in an experimental nursery located in Mokra Lewa, near Skierniewice, central Poland. However, this paper presents only the results from the two years (2011-2012) of the study, because since the very wet weather of 2013 caused more than 50% mortality among the experimental trees. The soil in which the trees grew was a podzolic soil. Soil analysis showed the pH of 6.1, and the mineral content at following levels: N (0.07% ADW), K (6.7 mg/100 g of soil), P (3.9 mg/100 g of soil), Mg (3.4 mg/100 g of soil) [20].

Rootstocks were planted every 25.0 cm within the row and 1.0 m between rows. For three consecutive years before establishing the nursery, cereal crops had been grown, and in the year preceding the planting of rootstocks, mustard plants had been ploughed in as green manure. The experiment was set up in a randomized block design with four replications, each repetition had 10 plants. In the first year (2010-2012) of running the nursery, the treatments were applied to cherry rootstocks (*Prunus mahaleb* L.) and to M 26 apple rootstocks [21]. In the second years (2011-2013), rootstocks were grafting by chip budding method, with using such cultivars as "Debreceni Bötermö" for sour cherry and to apple-"Topaz".

The following treatments were applied:

(1) No treatment (control);

(2) Micosat F12 WP (CCS Aosta Srl, Italy)—microbial inoculum consisting of mycorrhizal fungi (*Glomus mosseae* and *G. intraradices*) and plant growth promoting bacteria (*Pseudomonas fluorescence* and *Bacillus subtilis* strains). The product contains 40% C, 0.15% N, 431 mg·kg^{-1} P and 9,558 mg·kg^{-1} K, and has a granular consistency. Micosat was applied to the soil at a dose of 10 g·m^{-2} (100 kg·ha^{-1}) at planting;

(3) Chemical NPK application at a dose of 17.64 g·m^{-2} NH_4NO_3, 6.52 g·m^{-2} triple super phosphate, and 16.0 g·m^{-2} K_2SO_4, equivalent to 60 kg·ha^{-1} N, 30 kg·ha^{-1} P, and 80 kg·ha^{-1} K;

(4) Fertigo (Ferm-O-Feed, the Netherlands)—granulated manure containing 55% C, 1% N, 0.3% P and 1% K; besides these, also microelements and soil micro-organisms. The product was applied at a dose of 150 g·m^{-2} (1500 kg·ha^{-1}), equivalent to 45 kg·ha^{-1} N, 13 kg·ha^{-1} P and 17 kg·ha^{-1} K;

(5) Humus UP (Ekodarpol, Poland)—an extract from vermicomposts, containing 0.65% C, 0.03% N, 30.8 mg·kg^{-1} P and 4,535 mg·kg^{-1} K. The product was applied to the soil as a 2% solution (2 mL·m^{-2}) (20 L·ha^{-1});

(6) Humus Active + Aktywit PM (Ekodarpol, Poland)—an extract from vermicomposts based on a product derived from molasses. Humus Active is a soil improver with active humus and population of beneficial microorganisms, containing 0.78% C, 0.03% N, 1,050 mg·kg^{-1} P and 4,119 mg·kg^{-1} K. Aktywit PM is a soil improver containing 20.5% C, 0.92% N, 81.2 mg·kg^{-1} P and 42,990 mg·kg^{-1} K. (Humus Active was applied to the soil as a 2%

solution (2 mL·m^{-2}) (20 L·ha^{-1}), and Aktywit PM was applied to the soil as a 1% solution—1 mL·m^{-2} (10 L· ha^{-1}));

(7) BioFeed Quality (Agrobio Products B.V., the Netherlands)—an extract from several seaweed species reinforced with humic and fulvic acids, containing 0.6% C, 0.07% N, 32.6 mg·kg^{-1} P, applied to the soil as a 0.5% solution (0.5 mL·m^{-2}) (5 L·ha^{-1});

(8) BioFeed Amin (Agrobio Products B.V., the Netherlands)—an extract of vegetal amino acids, containing 1.12% C, 0.14% N, 347 mg·kg^{-1} P. The product was applied to the soil as a 0.5% solution (0.5 mL·m^{-2}) (5 L·ha^{-1});

(9) Vinassa (Józefów, Limited Liability Company, Poland)—molasses residue from yeast production, containing 12.0% C, 1.86% N, 949 mg·kg^{-1} P, 17,615 mg·kg^{-1} K. The product was applied to the soil as a 0.5% solution (0.5 mL·m^{-2}) (5 L·ha^{-1});

(10) Florovit Eko (Inco-Veritas, Poland)—product contains lignite, potassium sulphate (5% K), phosphorus (3% P), dolomite, bentonite, and molasses. It was applied to the soil at 150 g·m^{-2} (1,500 kg·ha^{-1}).

All the preparations except for Micosat, NPK and granulated manure (Fertigo) were applied twice during the growing season: the first time in the rootstock nursery at the end of May when the rootstocks started active growth, and again in the middle of June. Similar, the following year: first in the nursery of maiden trees in mid-May and again at the beginning of June. Micosat was applied to the soil by hand to a depth of about 15 cm. Following application of the preparations, the soil around the plants was thoroughly mixed using hand hoes each time. Protection of plants against pests and diseases was carried out by means approved for use in organic farming, and weeds were destroyed mechanically.

In the autumn, before digging up the trees from the nursery, measurements of their trunk diameter at a height of 30 cm aboveground and of their height were taken; the number of branched trees and the length and number of lateral shoots were recorded. Only the lateral shoots longer than 3 cm were included in the measurements.

The data obtained were processed statistically using a two-way analysis of variance for a randomized block design. Multiple comparisons of the means for the combinations were performed with Tukey's test at a significance level of $P = 0.05$.

3. Results

The effects of the use of organic fertilizers and mycorrhizal fungi on the morphology of apple and sour cherry maidens are shown in Tables 1-4.

Micosat applied to the soil in 2011 in the control combination with apple maidens that had not been previously fertilized gave significantly increased the trunk diameter (Table 1). Micosat in combination with NPK application had no effect on the above parameter. A significant increase in the trunk diameter of apple maiden trees under the influence of Micosat was found after fertilizing them with the preparations of Humus Active + Aktywit PM, Vinassa, and Florovit Eko. Micosat had a significant effect on the height of the maidens only in the combinations where Humus Active + Aktywit PM and Vinassa were used for fertilization. The effect of Micosat on the number of branched maidens was not significant. The preparation increased the number of lateral shoots in maiden apple trees in all the combinations of organic fertilization. By contrast, when combined with mineral fertilization, Micosat gave a worse result than fertilization with NPK only. Micosat significantly increased the total length of lateral shoots of maiden apple trees only where Florovit Eko was used.

In the second year of the study (Table 2), Micosat had no effect on the trunk diameter of maiden apple trees, but was found to stimulate significantly their growth in height. In combination with Fertigo manure, Humus UP, BF Quality, BF Amin and Vinassa, the mycorrhizal preparation had also a favourable effect on the growth of the apple maidens. However under

Table 1 Effect of various fertilization treatments on plant growth parameters of apple cv. "Topaz" maiden trees grown in an organic nursery, 2011.

Treatment	Trunk diameter mm	Tree height cm	Number of branched trees %	Number of lateral shoots#	Total length of lateral shoots# cm
Control**	10.5a*	97a	91.5a	3.2ab	17a
Micosat	12.4e-g	106a-c	90.0a	4.0c-f	15a
NPK	13.4hi	106a-c	99.5a	5.4hi	99h
NPK + Micosat	12.0c-f	111b-e	90.0a	3.6a-d	15a
Fertigo Manure	12.9f-h	113b-f	97.3a	4.3d-f	58de
Fertigo Manure + Micosat	14.3j	118d-f	88.8a	5.5hi	49bc
Humus UP	13.1g-i	121e-g	99.8a	3.4a-c	61ef
Humus UP + Micosat	11.4b-d	122fg	84.5a	5.3g-i	58de
Humus Active + Aktywit PM	11.4b-d	116c-f	100a	3.5a-c	51cd
Humus Active + Aktywit PM + Micosat	12.5e-g	134h	90.0a	5.5hi	49bc
BF Quality	11.0ab	114c-f	98.5a	3.1a	41b
BF Quality + Micosat	10.8ab	111b-f	89.8a	6.0ij	48bc
BF Amin	12.1d-f	109b-d	100a	5.2g-i	69f
BF Amin + Micosat	11.2a-c	103ab	92.5a	4.8f-h	58de
Vinassa	12.2d-f	117d-f	99.3a	3.9b-e	47bc
Vinassa + Micosat	13.9ij	130gh	93.5a	5.6i	48bc
Florovit Eko	11.9c-e	138h	100a	4.6e-g	54c-e
Florovit Eko + Micosat	13.6h-j	136h	97.3a	6.5j	83g

#—shoots longer than 3 cm, **—control (no fertilization); *—values that do not differ from each other are designated in each column by the same letters, according to Tukey's test at a significance level of P = 0.05.

Table 2 Effect of various fertilization treatments on plant growth parameters of apple cv. "Topaz" maiden trees grown in an organic nursery, 2012.

Treatment	Trunk diameter mm	Tree height cm	Number of branched trees %	Number of lateral shoots#	Total length of lateral shoots# cm
Control**	10.0a*	84a	81.8a	2.1a	13a
Micosat	11.0a	94b	95.0b	5.1f-h	41d
NPK	13.1a	103cd	97.5b	3.2ab	23bc
NPK + Micosat	13.2a	98bc	93.8b	3.7b-d	27c
Fertigo Manure	12.7a	106de	98.8b	3.3bc	22bc
Fertigo Manure + Micosat	13.7a	114f-h	95.0b	4.6d-f	44d
Humus UP	11.9a	107d-f	99.8b	3.8b-e	41d
Humus UP + Micosat	12.2a	114gh	99.3b	5.8gh	39d
Humus Active + Aktywit PM	11.5a	103cd	99.5b	3.0ab	20ab
Humus Active + Aktywit PM + Micosat	11.4a	104cd	95.8b	5.1f-h	39d
BF Quality	13.2a	107de	100b	4.4c-f	97h
BF Quality + Micosat	13.6a	112e-g	99.8b	6.1h	65f
BF Amin	13.1a	103cd	100b	5.0e-g	52e
BF Amin + Micosat	13.9a	120h	100b	5.9gh	63f
Vinassa	13.5a	107d-f	100b	3.0ab	24bc
Vinassa + Micosat	14.0a	116gh	99.5b	4.0b-f	37d
Florovit Eko	13.3a	106de	100b	3.1ab	40d
Florovit Eko + Micosat	13.7a	107d-f	99.3b	7.5i	75g

Explanations the same as below Table 1.

NPK mineral fertilization it tended to inhibit growth. The number of branched maidens increased under the influence of Micosat only when compared to the control. Micosat significantly stimulated the number of lateral shoots of maiden apple trees except to NPK mineral fertilizer combination, Fertigo manure and Vinassa preparations. Micosat favourably affected the total length of lateral shoots not only in the combination where it was applied alone, but also where Fertigo granular manure and the preparations BF Amin, Vinassa and Florovit Eko were used. However, in combination with the preparation BF Quality, its presence was of no significance for plant growth.

In sour cherry, in 2011, Micosat stimulated tree trunk diameter growth in all the experimental combinations except for Humus UP and BF Quality (Table 3). A significant increase in the height of maidens in Micosat presence was observed in all fertilization combinations except for those with NPK and Florovit Eko. However, Micosat had no effect on the number of branched trees in the nursery. In the combination where NPK was used to fertilize sour cherry maidens, Micosat did not enhance the formation of lateral shoots. A significant increase in the number of lateral shoots associated with its presence was found only where the maidens were fertilized with Fertigo manure and preparations BF Amin and Vinassa. A significant increase in the total length of lateral shoots was observed under Micosat treatment as well as in all the combinations of organic fertilization involving this product with exception to Humus UP and NPK mineral fertilization.

In 2012, Micosat stimulated growth in thickness of the trunk of sour cherry maidens. When combined with BF Amin it also had a positive effect on tree trunk diameter. In NPK-fertilized plots, growth of the maidens was the same as control trees (Table 4). Weaker growth (tree height) under Micosat treatment was observed when fertilization was with NPK and with the organic preparations Humus UP, BF Quality and Vinassa. Micosat had no effect on the number of

Table 3 Effect of various fertilization treatments on plant growth parameters of sour cherry cv. "Debreceni Bötermö" maiden trees grown in an organic nursery, 2011.

Treatment	Trunk diameter mm	Tree height cm	Number of branched trees %	Number of lateral shoots#	Total length of lateral shoots# cm
Control**	13.3a	124a	97.0a	4.0a	191a
Micosat	15.9de	141bc	100a	5.5b-d	309j
NPK	15.1b-d	148c-f	100a	6.0d-f	280fg
NPK + Micosat	15.9de	151d-f	99.8a	5.1bc	271ef
Fertigo Manure	14.8bc	142b-d	100a	6.0d-f	239c
Fertigo Manure + Micosat	17.7g	163gh	100a	7.4g	388m
Humus UP	16.4e	135b	100a	6.0d-f	353l
Humus UP + Micosat	16.8e-g	156e-g	99.5a	6.5f	345l
Humus Active + Aktywit PM	14.5b	156e-g	100a	5.4b-d	267e
Humus Active + Aktywit PM + Micosat	17.4fg	166h	100a	5.3bc	325l
BF Quality	15.0b-d	140bc	100a	6.2ef	263de
BF Quality + Micosat	15.0b-d	164gh	99.8a	6.0d-f	289gh
BF Amin	14.1ab	144b-d	100a	5.8c-e	255d
BF Amin + Micosat	16.4ef	165gh	100a	7.5g	299i
Vinassa	14.4b	138bc	100a	5.0b	217b
Vinassa + Micosat	15.8c-e	156f-h	99.5a	7.6g	294hi
Florovit Eko	15.1b-d	151d-f	100a	5.4b-d	237c
Florovit Eko + Micosat	16.4ef	146c-e	99.8a	6.0d-f	254d

Explanations the same as below Table 1.

Table 4 Effect of various fertilization treatments on plant growth parameters of sour cherry cv. "Debreceni Bötermö" maiden trees grown in an organic nursery, 2012.

Treatment	Trunk diameter mm	Tree height cm	Number of branched trees %	Number of lateral shoots#	Total length of lateral shoots# cm
Control**	10.5a	116a	97.0a	2.9a	144a
Micosat	13.9ef	133de	98.8a	4.4bc	186b
NPK	12.7bc	130cd	99.8a	6.5gh	270h
NPK + Micosat	10.2a	121ab	99.0a	4.0b	154a
Fertigo Manure	12.2b	125bc	100a	4.8cd	248ef
Fertigo Manure + Micosat	12.7b-d	131c-e	99.8a	4.8cd	261gh
Humus UP	12.5b	136d-f	100a	6.2f-h	351k
Humus UP + Micosat	12.3b	121ab	99.5a	5.1de	210c
Humus Active + Aktywit PM	13.8d-f	141f	100a	7.3i	301i
Humus Active + Aktywit PM + Micosat	14.0f	137ef	99.5a	9.0k	338j
BF Quality	13.7c-f	135d-f	100a	6.6hi	299i
BF Quality + Micosat	12.2b	125bc	100a	8.0j	307i
BF Amin	12.3b	131c-e	100a	4.8cd	223cd
BF Amin + Micosat	14.8f	132c-e	99.3a	8.0j	311i
Vinassa	12.9b-e	129cd	100a	5.8e-g	253fg
Vinassa + Micosat	12.8b-e	121ab	100a	5.6ef	269h
Florovit Eko	12.2b	125bc	100a	4.7b-d	221c
Florovit Eko + Micosat	12.6bc	120ab	100a	7.3i	235de

Explanations the same as below Table 1.

branched maidens, but significantly stimulated an increase in the number of lateral shoots, except for combinations with NPK and Humus UP.

4. Discussion

The scientific literature contains many reports on the effects of mycorrhizal fungi on growth and fruiting of horticultural crops such as strawberry [1, 2, 8-10]. Very few publications are available on the effect of mycorrhiza on growth and development of rootstocks and young fruit trees in organic nurseries, and later in orchards [4, 6, 16, 17]. Symbiosis between crop plants and mycorrhizal fungi is commonly known to exist. There is little information available on the effectiveness of mycorrhization in the presence of a specific group of organic fertilizers, or mineral fertilizers. No one questions that young trees in organic nurseries need nutrients for growth [14, 17]. Explanation is required, however, whether there is synergism or antagonism between the positive action of mycorrhizal fungi and that of a specific type of biofertilizers, or perhaps an indifferent response by plants to their presence [3, 10]. Only a partial answer to this question is given by the results of the present work. When mineral fertilization was used, the effectiveness of mycorrhizal fungi in the nursery of maiden apple trees was low or almost undetectable. This effect had been confirmed by previous studies carried out at the Research Institute of Horticulture in Skierniewice. Sas Paszt et al. [2] showed negative effects of NPK fertilization on the formation of mycorrhizas on the roots of fruit plants and the reduction in biodiversity and populations of beneficial soil microflora. Thus the low effectiveness of mycorrhiza is caused by the negative effect of NPK fertilization on the development of soil microflora and by the sufficient availability of NPK minerals under mineral fertilization. The effectiveness of mycorrhiza was also negligible when vermiculite (Humus UP) was used as a fertilizer. On the other hand, in the presence of manure or biopreparations such as BF Amin or Vinassa, stimulation of growth of young trees under the influence of mycorrhiza was clearly evident, which is consistent with previous studies conducted by Grzyb et al. [13, 21] and Sas Paszt et al. [2]. Mycorrhization had no effect on the growth of sour cherry maidens in the presence of the fertilizer Florovit Eko. In apple, by contrast, the joint action of mycorrhization and Florovit Eko, in terms of the intensity of tree growth and development, was significant.

There are reports in literature how the quality of trees at planting affects their subsequent growth and fruiting [15, 22, 23]. The reports emphasize that young trees to be used for establishing an orchard should have a specific height and trunk thickness, and be adequately branched. The authors state that trees already branched in the nursery produce flower buds early in the orchard, and thus guarantee high yields. They agree that every one-metre length of lateral branches bears two kilograms of apples in the second year after planting. In practice, the productivity is generally greater than 2 kg of apples per 1 m of lateral branches. Young trees for planting should thus not only be branched, but also have a sufficiently large number and overall length of lateral branches.

The authors' own studies have shown that mycorrhization in the soil environment does not always enhance the biological activity of the biopreparations used. It should also be mentioned that the resulting positive effects may differ considerably in different species of fruit trees. Application of mycorrhizal fungi can, however, be a factor contributing to the overall development of fruit plants, especially during their propagation in the organic nursery [14, 21].

Studies and observations carried out at the Research Institute of Horticulture in Skierniewice indicate much greater effectiveness of mycorrhization in plantings of young plants in the early stages of vegetative growth than in the later stages of their growth [2].

In the next stage of research, particular attention will be paid to the impact of treatments used in the nursery on the development of the root system maidens organically grown.

5. Conclusions

Micosat markedly stimulates the growth and development of apple ('Topaz'/M 26) and sour cherry ('Debreceni Bötermö'/*Prunus mahaleb*) maiden trees in the organic nursery.

The effect of the mycorrhizal preparation Micosat on the development of maiden apple and sour cherry trees previously fertilized with mineral fertilizers (NPK) is not significant.

In combination with organic fertilization, the presence of Micosat favours the development of apple and sour cherry maidens. The most effective in this process are: for apple—Fertigo granular manure, Vinassa and Florovit Eko, and for sour cherry—BF Quality, BF Amin and Humus Active + Aktywit PM.

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