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Influence of Biopesticides and Natural Preparations on the Regulation of Diseases and Pests in the Ecological Protection of Tomatoes (*Lycopersicon esculentum* L.) and Cucumbers (*Cucumis sativus* L.)

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Authors' contributions

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

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ABSTRACT

Organic agriculture is increasingly present on farms in response to environmental pollution and food safety. However, organic farmers have a big production risk due to frequent plant diseases. This research was conducted to compare the influence of preparations for plant protection allowed in organic agriculture and phytopharmaceutical preparations which are used in conventional agriculture and observe their effect in regulation of pests and diseases in tomato and cucumber production in an open field. The research included the tomato variety „Oxheart", and the cucumber variety „Long green". The time of research was four months from planting to harvest (period: May 2020. – August 2020.) until more than 70% of vegetation was completed. The tests were performed at a location in the rural part of the town of Živinice (Tupković) in an open field with an altitude of 250 m, during the vegetation period of 2020. The tests were performed on two separate plots. The plot where phytopharmaceutical (conventional) preparations were used, was physically separated from the plot where preparations allowed in organic agriculture were used. Temperature measurement was performed using a TFA Dostmann thermometer (-25ºC to + 50ºC) and it was set at a height of 2 meters in the open field. The amount of rainfall was monitored with the TFA

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Dostmann rain gauge. In the tests with organic plant protection, the nettle solution and preparation with active substance *Bacillus thuringiensis* for pests was used, and for plant diseases, the milk+soda solution and copper-oxide were used. In the tests with conventional protection, preparations with active substances acetamiprid and imidacloprid for pests were used, and the diseases were treated with mancozeb+copper and metalaxyl-M+mancozeb. After treatments, the onset of diseases was monitored. To conclude, the conventional insecticides gave better results in the regulation of pests in comparison to organic preparations, and in the case of organic preparations, the nettle solution had a better effect. Plant protection with copper-oxide gave the same results as using conventional preparations in disease regulation. This shows that plant protection is possible in organic production, but preparations must be applied more often and in a timely manner.

Keywords: Organic agriculture; biotechnical measures; biological control.

1. INTRODUCTION

The biggest obstacle for farmers in organic agriculture is the limited and insufficiently researched plant protection, considering that diseases or pests can completely destroy the crops. An organic protection system is defined by the law of organic agriculture and a certification accredited body. It doesn't necessarily mean not using any safeguards, but using preparations that are approved by law and the certification body whose impact on people's health has been tested. Every country that has organic production should have a list of permitted plant protection products and fertilizers.

The essence of plant protection in organic agriculture is to maintain the level of pests below the threshold of economic damage [1], which is achieved by preventive, agrotechnical, and biotechnical measures. These measures are applicable through accurate crop rotation and mix of crops, selection of plots, preservation of useful organisms which will regulate pests, the use of effective microorganisms (bacteria, viruses), and lastly the use of biopesticides and physical protection methods [2]. Organic farmers rely on natural pest control (for example biological control, using plants with pest control properties) rather than synthetic pesticides that are known to kill beneficial organisms which can also pollute water and soil.

These methods of protection strive to obtain healthier products without harmful effects on the environment considering that biopesticides degrade quickly in plants or soil. However, the difference between biopesticides and conventional pesticides is that they don't have rapid effectiveness against pests and diseases. The use of biopesticides is possible only after the approval of the inspector of the relevant certification body for organic production.

Tomatoes and cucumbers are economically the most important vegetables in Bosnia and Herzegovina because of high market demand. Intensive exploitation and conventional breeding of these two species have affected their reduced resistance to external factors, especially the lower immunity to diseases and pests. While growing in the open field, cucumber plants are exposed to attacks by different diseases and pests which cause large damages every year. The most significant diseases are downy mildew (*Pseudoperonospora cubensis*), angular leaf spot (*Pseudomonas syringae pv lachrymans*), powdery mildew (*Erysiphe cichoracearum*), fusarium wilt (*Fusarium oxysporum*). Some pests worth mentioning are aphids and red spider mites (*Panonychus ulmi*). In the case of tomatoes, late blight (*Phytophthora infestans*) is the most common and the most harmful disease which can completely destroy crops and cause tomato plants to become addicted to fungicides [3]. Losses caused by Phytophthora infestans can be up to 7% in tomato production for fresh consumption [4]. Fruit infection can be 41-100% in open field production. Soil degradation and loss of organic matter caused an increased incidence of root disease. High nitrogen concentrations and unbalanced fertilization caused larger susceptibility of root and shoot system of a plant on diseases [5].

Compared to conventional plant protection, organic protection system have a lot of advantages like larger plant diversity in time and space, accurate crop rotation (which leads to a greater organic matter content in the soil), higher biomass, better diversity and activity of soil microorganisms, increased soil capacity for water, improved cation exchange capacity. These differences between organic and conventional protection have resulted in

differences in the occurrence and intensity of plant diseases and pests [6].

The organic protection system is a big challenge for farmers, especially at cultivation in the open field where plants are completely exposed to climate changes and are susceptible to biotic and abiotic stresses, diseases, and pests. Aim of this work is to give farmers more data about organic protection method comparing it with using pesticides and see the difference in yield at the end of vegetation.

2. MATERIALS AND METHODS

The research examined the influence of conventional pesticides, biopesticides, and natural preparations in plant protection. The location was set in the rural part of the town Živinice (Tupković) in an open field with an altitude of 250 meters. The tomato variety „Oxheart“ and cucumber variety „Long green“ were tested, while the cucumber was grown with a support net. The time of research was four months from planting to harvest (period: May 2020. – August 2020.) until more than 70% of vegetation was completed.

The tests were performed on two separate plots. The plot that used phytopharmaceutical preparations was physically separated from the plot that used preparations allowed in organic agriculture. Each of these two plots had three tests for tomato variety and three tests for cucumber variety. In two tests, different protection programs were applied and the third test was a control. The label of every test is shown in Table 1. In the plot with organic plant protection, the natural preparations and biopesticides were used in two different tests. The aim was to perceive which of those two plant protections had better results. From natural preparations, the nettle solution was used in the proportion of 1 kg nettle leaf + 10 liters of water for pests, whereas the milk + soda solution in the proportion of 1 liter of milk + 30 g of soda + 5 liters of water was used for diseases. From biopesticides, the preparation „Kraken“ with active substance *Bacillus thuringiensis* for pests and the preparation „Nordox“ with active substance „copper-oxide“ for diseases were used. In the plot with conventional plant protection, phytopharmaceutical pesticides with different active substances were used in two tests. The first test included the application of insecticide „Boxer“ with active substance imidacloprid for pests and the preparation „Ridomil Gold MZ“ with active substance metalaxyl-M + mancozeb for diseases. The second test included treatments of insecticide „Mospilan“ with active substance acetamiprid for pests and the preparation „Cuprofix“ with active substance mancozeb+copper for diseases. The concentration of treatments is shown in Table 2. The time and the total number of treatments are shown in Table 3. The following diseases were monitored: fusarium wilt (*Fusarium oxysporum*), late blight (*Phytophthora infestans*), early blight (*Alternaria solani*), downy mildew (*Pseudoperonospora cubensis*). With regard to pests, the occurrences of aphids like the green peach aphid (*Myzus persicae*) and the black bean aphid (*Aphis fabae*) were monitored. *Myzus persicae* was present on tomato plants and *Aphis fabae* was present on cucumber plants. Infection of diseases and pests was natural in the open field.

Morphometric and physical analysis of the fruits included three parameters: shape, color, and weight. The calculation of daily average temperature was performed according to the formula: T1 = (T7 + T14 + 2xT21) / 4 [7].The temperature measurement was performed using the TFA Dostmann thermometer (-25º C to + 50ºC) and it was set at a height of 2 meters in the open field. The temperature was measured three times a day at 07:00 AM, 02:00 PM, and 09:00 PM. The amount of rainfall was monitored with the TFA Dostmann rain gauge.

Table 1. Labels of tests

Culture	Label	Interpretation	Number of plants
Cucumber	K-Pp	Test treated with natural preparations	30
Cucumber	K-Bp	Test treated with commercial biopesticides	30
Cucumber	K1	Control test of cucumber in organic protection	30
Cucumber	K-I	Test treated with conventional pesticides (1)	30
Cucumber	K-II	Test treated with conventional pesticides (2)	30
Cucumber	K2	Control test of cucumber in conventional protection	30

Culture	Label	Interpretation	Number of plants
Tomato	P-Pp	Test treated with natural preparations	30
Tomato	P-Bp	Test treated with commercial biopesticides	30
Tomato	K3	Control test of tomato in organic protection	30
Tomato	P-I	Test treated with conventional pesticides (1)	30
Tomato	P-II	Test treated with conventional pesticides (2)	30
Tomato	K4	Control test of tomato in conventional protection	30

Table 2. Active substances and treatment concentrations

Culture	Label of test	Active substance (AS)	Concentration of AS in preparation	Dosage / Concentration of treatments
Cucumber	K-Pp	Milk + soda Nettle leaf solution	-	10% + 0,4% 10%
Cucumber	K-Bp	Copper(I)oxide *Bacillus thuringiensis*	75% copper 160 g/l	0,15% 1,5 kg/ha
Cucumber	K1	- *control in organic protection*	-	-
Tomato	P-Pp	Milk + soda Nettle leaf solution	-	10% + 0,4% 10%
Tomato	P-Bp	Copper(I)oxide *Bacillus thuringiensis*	75% copper 160 g/l	0,15% 1,5 kg/ha
Tomato	K3	- *control in organic protection*	-	-
Cucumber	K-I	Mancozeb + Copper Acetamiprid	300 g/kg + 120 g/kg 20%	4-5 kg/ha 0,0125%
Cucumber	K-II	Metalaxyl-M + Mancozeb Imidacloprid	38,8 g/kg + 640 g/kg 200 g/l	1,5-2,5 kg/ha 250 ml/ha
Cucumber	K2	- *control in conventional protection*	-	-
Tomato	P-I	Mancozeb + Copper Acetamiprid	300 g/kg + 120 g/kg 20%	4-5 kg/ha 0,0125%
Tomato	P-II	Metalaxyl-M + Mancozeb Imidacloprid	38,8 g/kg + 640 g/kg 200 g/l	1,0-2,5 kg/ha 250 ml/ha
Tomato	K4	- *control in conventional protection*	-	-

3. RESULTS AND DISCUSSION

The research included two test areas that are important for organic agriculture:

1. Influence of commercial biopesticides on the regulation of diseases and pests,
2. Influence of natural preparations on the regulation of diseases and pests.

In tests where the plant protection was applied in accordance with the principles of organic production, the commercial biopesticides available to producers on the market of Bosnia and Herzegovina were used. Natural preparations were obtained from plants found in nature and allowed for the use in organic plant protection.

In tests with conventional plant protection, pesticides with two different protection programs and two different active substances for the same diseases or pests were used. The active substances of preparations and concentration of treatments are listed in Table 2.

Table 3 shows the time of treatment application, the total number of treatments, and the preparation withdrawal period.

Table 3. Time, number of treatments and withdrawal period

Treatment	Total number of treatments for tomato	Time of treatment for tomato	Total number of treatments for cucumber	Time of treatment for cucumber	Withdrawal period (WP)
Nettle leaf solution	14	Every 7 days	13	Every 7 days	No WP
Bacillus thuringiensis	6	11.05. 26.05. 10.06. 25.06. 09.07. 24.07.	3	21.05. 05.06. 20.06.	No WP
Acetamiprid	2	11.05. 21.05.	2	23.05. 02.06.	3 days
Imidacloprid	2	11.05. 21.05.	2	23.05. 02.06.	14 days
Milk + soda	14	Every 7 days	13	Every 7 days	No WP
Copper(I)oxide	4	17.05. 28.05. 08.06. 21.06.	2	17.05. 28.05.	14 days
Mancozeb + Copper	3	17.05. 28.05. 08.06.	3	17.05. 28.05. 08.06.	14 days
Metalaxyl-M+Mancozeb		17.05. 28.05. 08.06.		17.05. 28.05. 08.06.	7 days on open field

Table 4. Average monthly temperatures and total amount of rainfalls for period May-August 2020

Month	Average monthly temperature (°C)	Total amount rainfalls (mm)
May	15.63	72.50
June	19.89	223.00
July	22.43	50.00
August	25.00	61.00

The production success, the appearance of pathogens, and pests are greatly influenced by the amount of light, rainfalls, and the number of rainy days. The optimal amount of water for tomato is 450-500 mm, while the amount of water for cucumber is higher (about 800 mm during the growing season) [8] which was supplemented by irrigation in this research. The problem with rainfalls was that in certain periods rainy weather lasted, while in others there was a drought, which caused floods. Observed by months, the total amount of rainfalls for May was 72.50 mm, June 223.00 mm, July 50.00 mm, and August 61.00 mm (Table 4). The highest daily rainfall was recorded on June 22nd2020 with 78 liters/m². In the monitored period, there were 33 rainy days.

The intensity of diseases and pests was observed every 7 days while monitoring plants' developmental stages and agroecological conditions. Biological insecticide with active substance *Bacillus thuringiensis* is intended primarily for the control of insects from order Lepidoptera or Coleoptera [9]. The efficiency has been proven on the insect *Tuta absoluta* [10]. Four *Bacillus thuringiensis* δ-endotoxins were found to exhibit low to moderate toxicity on the pea aphid (*Acyrthosiphon pisum*) in terms of mortality and growth rate, while one of them had a nontoxic exhibit at high rates [11]. The effect of *B. Thuringiensis* on handling *Myzus persicae* in tomatoes and *Aphis fabae* in cucumbers was almost as low as in control tests. The nettle solution gave better results, especially in the

reduction of *Myzus persicae* on tomatoes. The nettle solution has shown effectiveness in stopping the spread of aphid populations and slowed their growth and development but has not affected the mortality of existing insects. The results of other studies show that the nettle solution (in ratio 1:5) can reduce the fertility of Myzus persicae by 20% on average [12]. As expected, conventional insecticides gave the best results in reducing aphids, but they were also harmful to useful insects.

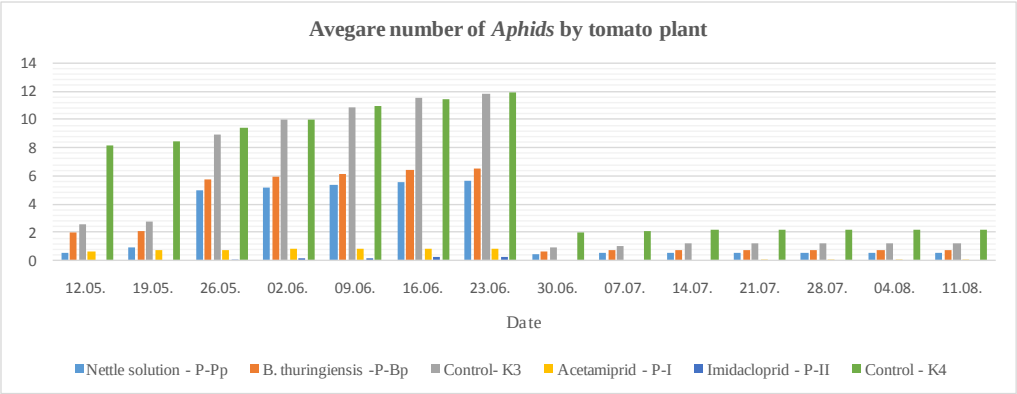

Fig. 1. Average number of aphids per tomato plant

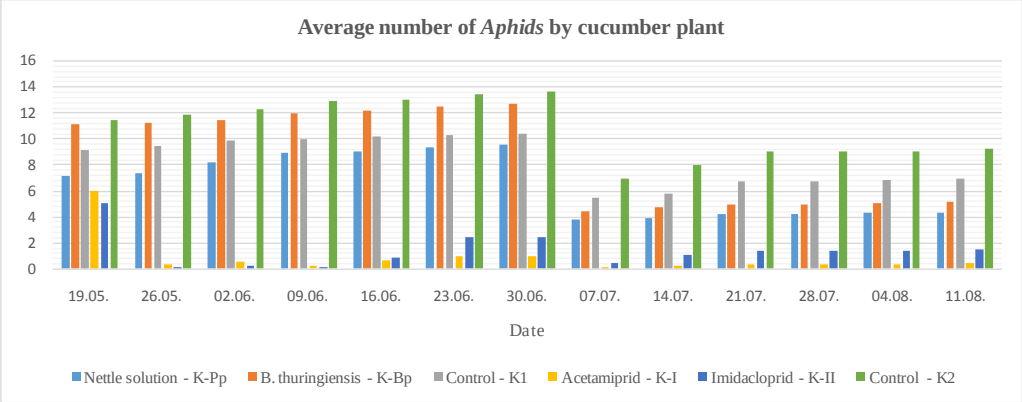

Fig. 2. Average number of aphids per cucumber plant

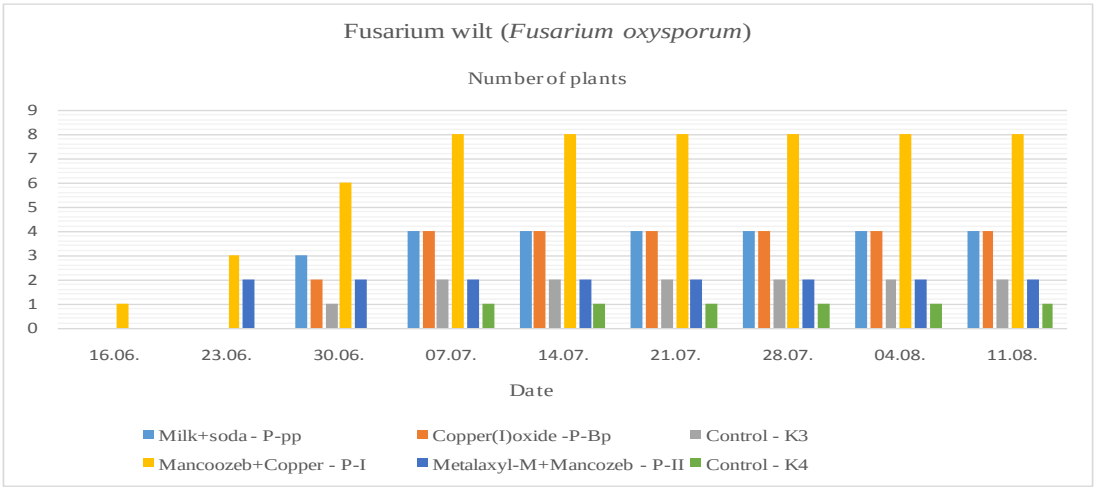

Fig. 3. Number of tomato plants infected with *Fusarium oxyporum*

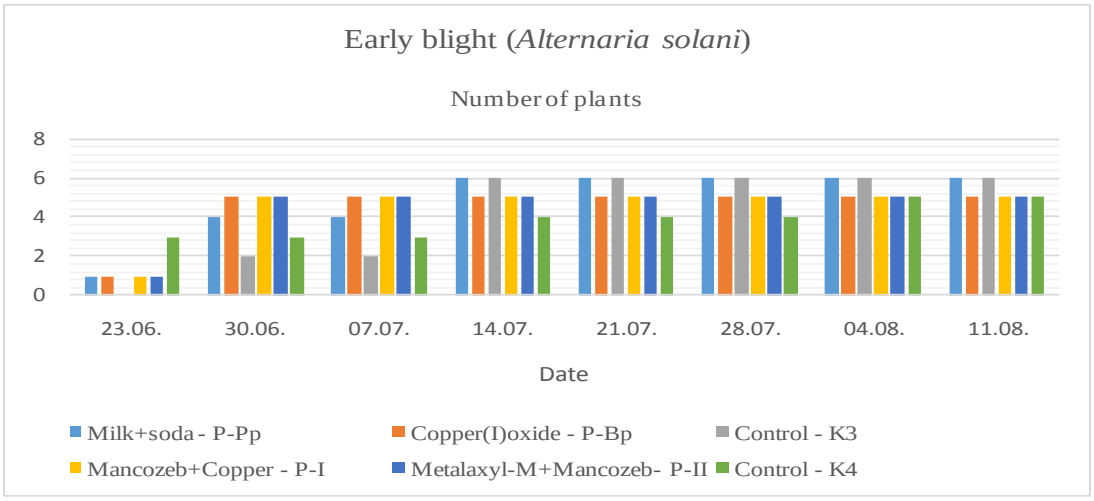

Fig. 4. Number of tomato plants infected with *Alternaria solani*

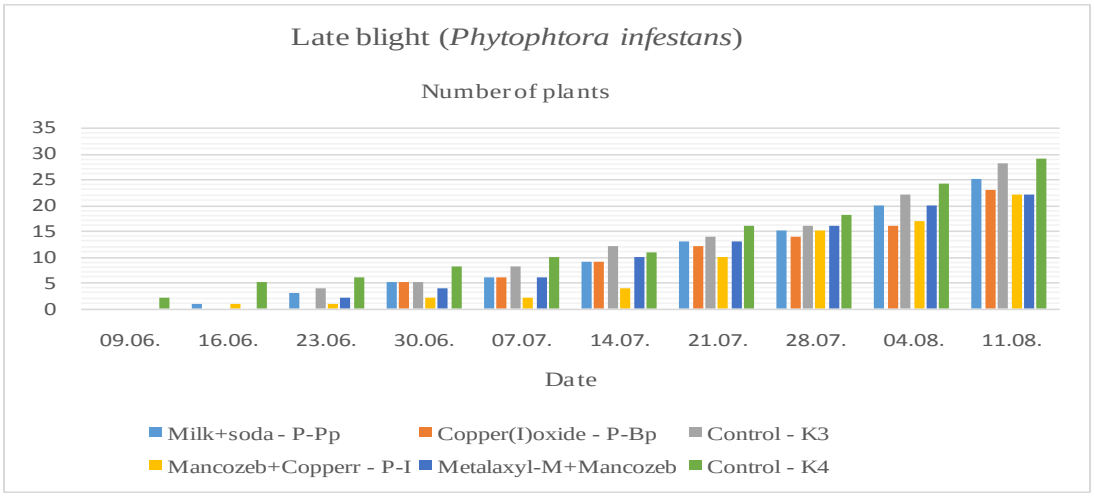

Fig. 5. Number of plants infected with*Phytophthora infestans*

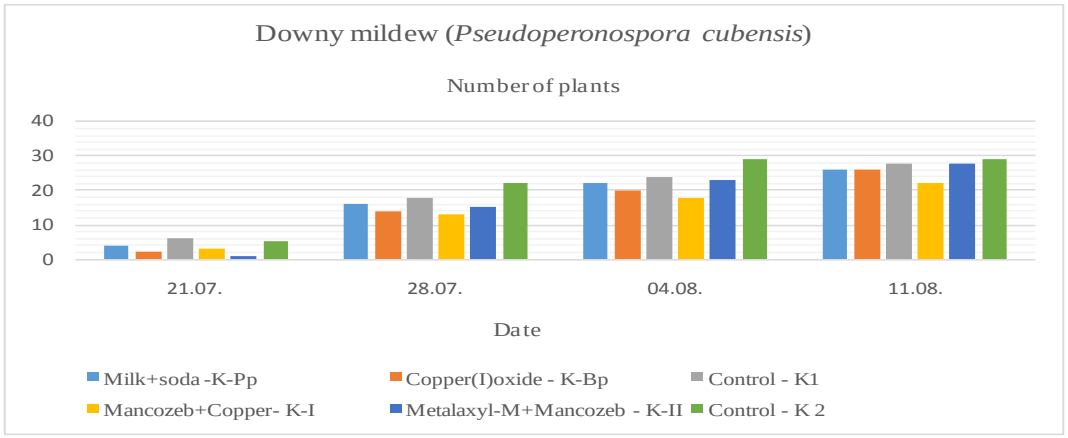

Fig. 6. Number of plants infected with *Pseudoperonospora cubensis*

Fusarium oxysporum appeared on tomato plants in the second half of June, primarily in tests treated with mancozeb + copper, where it caused the most damage. In the period of occurrence and further development *F. oxysporum*, the average daily temperature was 16.8ºC. Since the whole plant was affected and wilted, those plants had to be removed from the experiment to not be a source of infection to other plants. The first symptoms of early blight appeared at the end of June and the average daily temperature for occurrence and further development of this fungus was 16.9ºC. Symptoms of the disease appeared on several leaves and stems in the form of concentric circles. The highest development of this pathogen was in the period from 23rd June till 14th July, and after that, it began to stagnate.

Treatments of copper-oxide gave better results in organic protection, but the combination of milk and soda gave similar results. Milk and baking soda have been used in organic plant protection for a long time. Milk acts like glue and soda has an alkaline pH value which is unfavorable for many fungal pathogens. The efficiency of the milk+soda combination was 86,6% for *Fusarium oxysporum*, 80% for *Alternaria solani*, and 16,6% for *Phytophthora infestans*. The efficiency of copper-oxide was 86,6% for *Fusarium oxysporum*, 83,3% for *Alternaria solani*, and 23,3% for *Phytophthora infestans*. Similar research in the reduction of fusarium wilt was conducted on Chrysanthemums plants with nanoscale CuO and results were in the range from 32-55% [13]. Copper-oxide treatment in dosage 50 mg/L had efficiency of 54,28% in reducing *Phytophthora infestans* on potato plants in vitro conditions [14].

The first symptoms of cucumber downy mildew were manifested in control tests of conventional protection (K2) at the end of June. After that, the disease progressed and by the end of August, it infected all plants. Symptoms of the disease were manifested only on leaves but as infected leaves dried, the assimilation surface decreased, and the plant lost vital abilities. The average daily temperature for the appearance and development of this pathogen was 23ºC. For the infection by *Pseudoperonospora cubensis* leaves need to be moistened 90-100% [15]. At began of the infection the most resistance was at test treated with copper-oxide and comparing results with convencional protection method it's distinctly that protection in organic production gave the similar or the same results. Since the cucumber harvest began on 24th June and the first symptom was on 21st July, the downy mildew didn't completely affect the yield and harvest of cucumber, as was the case with tomatoes.

The yield of cucumber was in the range of 3.30-4.00 kg per plant in the organic system of protection, and 3.80-5.00 kg per plant in the conventional system of protection. Looking at the research in organic plant protection, the highest yield of cucumber had the test treated with commercial biopesticides which comes to 4.00 kg per plant.

Table 5. Average weight of fruit and total yield of cucumber

Labels	Average weight of fruit (gr)	Yield (gr / plant)
K-Pp	228.3	3500
K-Bp	232.5	4000
K1	180.9	3300
K-I	152.9	3800
K-II	169.1	5000
K2	150.4	3900

Table 6. Average weight of fruit and total yield of tomato

Labels	Average weight of fruit (gr)	Yield (gr / plant)
P-Pp	116.4	900
P-Bp	181.0	2000
K3	113.7	1400
P-I	100.0	1750
P-II	228.0	3800
K4	127.5	1900

The tomato had a quite low yield considering that plants diseased because of late blight (*Phytophthora infestans*) very soon after maturity. The yield of tomatoes in the organic system of protection was in the range of 0.9-2.00 kg per plant and 1.75-3.80 kg per plant for tests in the conventional protection system.

5. CONCLUSION

After the analysis of all parameters, the most important conclusions are:

1. Nettle leaf solution in concentration 10% applied every seven days during the vegetation had a better effect in reducing aphids (*Myzus persicae*; *Aphis fabae*) on tomato and cucumber varieties compared to the use of the commercial biopesticide based on *Bacillus thuringiensis*.
2. Copper (I) oxide in the concentration 0.15% applied two times during the vegetation showed better protection of cucumber against *Pseudoperonospora cubensis* compared to the treatment of natural preparations (milk + soda), but not in the comparison with conventional fungicides.
3. Copper (I) oxide in the concentration 0.15% applied four times during the vegetation showed better protection of tomato against *Alternaria solani* and *Phytophthora infestans*, compared to the treatment of natural preparations (milk + soda), and the protection gave the same results as the application of conventional fungicides.
4. Tests of tomato and cucumber treated with commercial biopesticides accomplished a larger yield compared to tests treated with natural preparations.

To summarize the results of this research, we can state that treating plants with preparations allowed in organic agriculture gives similar effectiveness in plant protection from economically consequential diseases and pests as treating plants with conventional pesticides. The difference is that natural preparations or biopesticides have to be applied more often in comparison to conventional pesticides. Also, the yield of both ways of protection was similar. This proves that plant protection in organic agriculture is possible and gives satisfactory yields and indisputable quality with adherence to agricultural techniques, well-organized protection, and favorable agroecological conditions. However, to reduce the risk of attacks by microorganisms or pests, it is recommended to grow plants in greenhouses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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