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Application of Propolis Extracts in Prevention of the Occurrence and Development of *Phytophthora infestans* in Ecological Agricultural Production of Tomatoes (*Solanum lycopersicum)*

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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 Agricultural Production is a special system of food production that exists in a rule-based, socially demanding, and environmentally constrained space within wider agriculture. It must meet social standards in key areas, such as sustainable use of natural resources, while minimally

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impacting local ecosystems throughout the growth cycle. The use of phytopharmaceutical substitutes is a core component, replacing conventional agriculture pesticides which have been shown to damage local ecosystems. In recent years, considerable research effort has been put into discovering alternative natural substitutes with pesticidal and antifungal properties.
The aim of this paper is to investigate the effects of propolis extracts on the growth and development of Phytopathological fungi, presenting an alternative to other phytopharmaceutical substitutes.
In this paper, the "American" tomato species are used throughout as it represents a common supermarket tomato variety. The following are the conclusions central to this paper: (1) preventative usage of alcoholic extracts controlls the likelihood of development of *Phytophthora infestans* and its subsequent growth while having a reduced environmental impact (2) a comparison of propolis extracts with other staple organic extracts found improvements in profitability, produce growth speed and energy requirement (3) produce yield was increased with the use of a propolis solution compared to other organic extracts, however, a combined alcoholic extract of propolis was found to have further benefits, specifically in the prevention and neutralization of aphids.

Keywords: Propolis; phytopathological fungus; prevention; organic agriculture; tomato; Phytophthora infestans.

1. INTRODUCTION

Organic agricultural production represents a special system of Crop production. It employs highly specific ecological practices tailored towards the preservation of natural resources, local ecosystems, and the overall environment in the long term. In order to ensure sustainability in agricultural production, a fine balance must be struck between the use of damaging pesticides, herbicides, and fungicides and the principles of organic agriculture. In conventional agriculture, the use of these chemicals is widespread in maximizing yields with the detrimental effect on local ecosystems often a side effect [1].

Little and Frost (2008.) state the key functional elements of the organic system as follows:

- Good management of soil, which leads to high fertility keeping a high concentration of microbiological activity.
- An effective and well-planned crop rotation leading to lower rates of disease, a balanced soil nutrition profile, and effectively combatting pests and weeds [2].

In recent years, a research priority in organic agriculture has been identifying natural plant disease control measures that use natural compounds such as alcoholic extracts of propolis. Their purpose is to prevent or limit the growth of fungal diseases, specifically phytopathogenic fungi [3], which can have a severe impact on crop yield and profitability.

Statistics from the 2009–2010 world harvest [4] suggest fungi-induced losses in five of the most important crops globally (rice, wheat, maize, potatoes, and soybean). In this complex scenario, it is clear that global warming and accompanying climate changes have resulted in increased incidences of many fungal diseases [5]. If those losses were mitigated, these crops would have been enough to feed 8.5% of the seven billion population in 2011. Furthermore, in a hypothetical event where these five crops were affected simultaneously, approximately 61% of the world's population would not have food [6]. Including all of the preventive measures, the right ways of dealing with pests and diseases, and discovering different extracts to fungal infestation will enable future generations to survive.

Tomato is a herbaceous plant from the family of *Solanaceae*. Tomatoes are native to Central and South America, from which it was introduced across Europe by the Spanish in the 16th century. Total production across the EU reached nearly 18MMT [7] in 2021 making up 46% of the global production [8]. Monoculture causes a lot of problems which include higher humidity, less ventilation, and better conditions for disease development especially fungus.

The use of alternative control methods that are efficient and have been proven on a regular basis, have low potential for environmental contamination; meaning they are safe for the local ecosystem, and do not cause damage to the health of the farmer/scientist have been the subject of various studies. Possible usable

materials are extracts of propolis, as they have a high potential to possess antimicrobial, antifungal, antioxidant, antiviral, and antiprotozoal activity [9]. The effective use of propolis extract in agriculture has been demonstrated through the control of bacteria and fungi *in vitro* conditions. Due to its antimicrobial properties propolis has been utilized by man for millennia, however, only recently has its use as an agricultural antimicrobial agent been tested, and is being used in agricultural production. The capacity of this product to activate plant defense mechanisms is highly promising, due to the large number of substances in its composition [10], by having a positive effect on plant vigor.

Phytophthora infestans is the most common disease which causes high losses in the production of tomatoes and other plants included in the family of *Solanaceae.* In the right conditions,the fungus spreads very fast and is able to destroy whole crops which were not treated with the right fungicides. *Phytophthora infestans* causes enormous economical damage, lowers plant yield by 65% and it is able to destroy whole crops in just 15 days. All other plants that are close by and are a part of the *Solanaceae* family such as potatoes, peppers, and others, can easily get infected by this fungus [11]. The main goal of every agricultural production is to have stable crops without diseases, pests, and weeds, in order to get a high income by having high plant yield. To get that goal before starting any agricultural food production it is needed to take into consideration the climate and good and bad plant neighbors, which helps a lot if it is planned from the beginning.

As the human population increases, the amount of food produced is very important. Unfortunately, there are other organisms out there that want to consume crops that are meant for humans. It is estimated that nearly 37% of all crops produced in the United States each year are destroyed by agricultural pests, which results in an economic loss of around $122 billion a year. Due to this high loss in food production, pesticides are often used to try to combat the problem. When pesticides are used, they do not always stay in the location where they are applied. They are very mobile and often move through water, air, and soil. Pesticide mobility can cause harm when in contact with other organisms and also disrupt the balance of the ecosystem. In many situations, when a pesticide is used, it also kills non-pest organisms. This can drastically alter the natural balance of the ecosystem. By removing non-pest organisms, the environment can be changed to favor the pest. Another major problem associated with pesticide use is bioaccumulation and biological magnification. Bioaccumulation is when a substance builds up in the body because the body does not have the proper mechanisms to remove it. Many synthetic pesticides are not degradable. Once they enter the body of an organism, they are permanently stored in the body tissue [12].

The pathogen *Phytophthora infestans* is a polyphagous fungus. It can infect potatoes, aubergine, tomatoes, and many more plants. Symptom soccur on leaves, stems, and fruit. The color and size of depigmentation depend on the place of infection and how long it took to notice them. Usually, lower-positioned leaves forma white form of sporangia on their back part. Eventually, with no treatment, all leaves can get infected and can fall off. On infected fruit on its bottom part symptoms occur as a black and red ring [13].

The chemical composition of propolis depends on the time and place from where it was collected, Fig. 1. It can vary in the amount of resin, oil, organic materials, type of metals, and concentration of flavonoids.Vitamins B1, B2, B6, C, E and minerals as silver, coper, mangan, iron, aluminium, pottasium can be found in propolis [14]. Flavonoids have a direct impact on immunomodulation and immunosuppression which occurs in plants as a result of infection with different pathogens, it represents a natural reaction of its immune system to remove a pathogen from an infected organism[15].

Fig. 1. Propolis

Lindenfelser (1967) analyzed propolis collected from different sources, and investigated its antibacterial activity against 25-39 bacterial species in vitro, and reported that propolis was effective in inhibiting *Bacillus larvae* [16].

Rosential (1989) showed that an alcohol solution with a concentration of propolis of 30% was effective in inhibiting *Bacillus cereus* and *Escherichia coli* [17].

Atiković et al. (2020) showed in their research that 0.20% and 0.15% alcohol extracts of propolis have a positive effect on inhibiting the appearance, growth and development of *Taphrina deformans* and *Plasmopara viticola* [18].

By working on the development of natural organic preparations in the prevention and suppression of diseases, although their effectiveness has been proven for a long period of time, research shows that different concentrations of solutions have a negative effect on the occurrence of diseases, while at the same time improving the condition of the plant.

2. MATERIALS AND METHODS

The experiment was conducted at Family Agricultural Household „Atiković“ in Tuzla at 508 meters altitude, starting from April 2022 until August 2022. Propolis extracts were made with propolis collected from *Apis mellifera* – a European honey bee from the city named Gunja in Croatia. Propolis extracts were prepared in 3 different concentrations according to the recommendation of Tringale [19], Table 1.

This experiment include the use of 0,20%, 0,15%, and 0,10% of propolis extracts concentrations. They have been carefully prepared in a couple of steps. The first step was freezing propolis and then grinding it into a fine powder. The second step is mixing fine propolis powder with 97% denatured alcohol and adding the appropriate amount of pure sulfur. These extracts as previously stated were prepared in three different concentrations, which were stored in conditions without any sunlight and in dark glass bottles. Extracts were stored for 20 days and on the last day they were filtered.

2.1 Impact of Alcoholic Extract of Propolis on *Phytophthora infestans*

The experiment was conducted *in vivo* conditions meaning the environment was not free from *Phytophthora infestans*. The total number of plants that were included in the experiment was 28 of which 12 of them were treated with alcoholic extracts of propolis, 13 plants were treated with other extracts allowed to be used in organic agricultural production, and the last 3 plants were used as a control on which non of the treatments were done, Table 2.

Table 1. Recommendation of Tringale [16], for usage of extracts

Fruit	Disease or pest	Usage of the propolis extract
Actinidija	Gray mold (*Botrytis)* Aphids	0,2% hydro-alcoholic solution + 0,3% wettable sulfur 2-3 treatments with the alcohol solution
Citrus fruits	Anthracnose (*Colletotrichum gloesporoides*) Fruit mold (*Phytophtora citrophtora*) Thyroid aphids (*Mytiloccocus beckii, Lepidosaphes gloverii* i dr.)	After removing the infested branches carry out 2-3 treatments with 0,2% hydro-alcoholic solution Treat fruits before or immediately after harvest with 0,1% alcohol solution and allow them to air dry Coat the infested branches with propolis oil
Olive	Olive fly (*Dacus oleae*) Cancer (*Pseudomonas savastanoi*) Thyroid aphids (*Lepidosaphes destefani, Lucaspis riccae*)	When the attack is not stopped in time, an alcoholic extract of 0,1% + wettable sulfur effectively destroys the eggs of the olive fly inside the fruit 2-3 treatments with 0,2% hydro-alcoholic solution Coat the infested branches with propolis oil
Peach	Curliness (*Taphrina deformans*)	At the appearance of the first symptoms, carry out repeated treatments with an alcoholic solution of propolis (0,2%) + wettable sulfur (0,35%)
Grapevine	Downy mildew (*Plasmopara viticola*)	According to meteorological conditions, carry out numerous treatments with hydro-alcoholic solution (0,2%) + wettable sulfur (0,3%)

Fruit	Disease or pest	Usage of the propolis extract
	Gray mold (*Botrytis cinerea*)	Carry out treatments with hydro-alcoholic solution (0,2%) + wettable sulfur (0,2-0,3%) according to meteorological conditions

Table 2. Interpretation of the experiment

Plant	Mark	Interpretation	Number of Plants
Tomato	0,10%	Treated with alcoholic solution	4
Tomato	0,15%	Treated with alcoholic solution	4
Tomato	0,20%	Treated with alcoholic extract	4
Tomato	S	Treated with a solution of milk + baking soda, nettle solution, detergent + cinnamon	13
Tomato	K	Not treated	3
Total			**28**

Application of the treatments wasper formed manually using a sprayer with a conical nozzle until plants were completely covered in propolis extracts. All of the plants in this experiment were seedlings without previous infection with *Phytophthora infestans* and on the plantation infection occurred every year.

3. RESULTS AND DISCUSSION

During the whole period of monitoring whether*Phytophthora infestans* is going to occur and develop on tomato plants, all preventive agro-technical measures were performed: pruning, fertilization, irrigation, cultivation of rows and inter-row space, removal of old leaves and placement of backrest. Special attention was paid to the fact that the infection caused by *Phytophthora infestans*can not be subsequently controlled and protection must be carried out preventively. Keep in mind it can cause major disaster by destroying all of the cultivated plants from the family *Solanaceae*, not just tomatoes. During this period temperatures and humidity were perfect for the development of this fungus (3-26°C and 9-90%), Table 3.

During the period beteen of April until August, all three alcoholic extracts of propolis proved to be effective in stoppingthe development of *Phytophthora infestans*. All three plants that were used as a control group died from being infected by this fungus because non of the preventive measures have been used on them. All three groups of the experiment were in close proximity to each other in that way the risk of infection was high. Treatments were done based on therecommendation of PIS „Vojvodina“, Table 4.

Propolis extracts are known for not being harmful to other living beings, because of that when applied there is no need for a waiting period (karenca). Atthe end of June (29th June) and the beginning of July there was a warning put out for *Aphidae* that was the main reason why nettle extracts and a mixture of detergent and cinnamon were used on a part of the plantation where alcoholic extracts of propolis were not used. This way was proven that propolis extracts have repellent properties on *Aphidae*, they never occurred on plants treated with alcoholic extracts of propolis.

3 plants that were used as a control for this experiment died in the middle of July, the infection was so strong that it killed them in a very short time. The first symptoms occurred on the 30th of June. As a result,the amount of fruit production was 0 kg/plant. Other 13 plants were treated with other extracts in order to compare the efficiency of alcoholic extracts of propolis with other extracts which are allowed to be used in organic agricultural production. On these plants fruit production was a little bit lower when compared with a group of plantsthat were treated with alcoholic extracts of propolis. The first symptoms occurred very late on the 8th of August (2 plants) and they were on the oldest leaves on the bottom of the plant, seen as depigmentation, and on the reverse side of leaves,a white coating of sporangia was noticed. Compared with controlled plants, non of these have been fully infected.

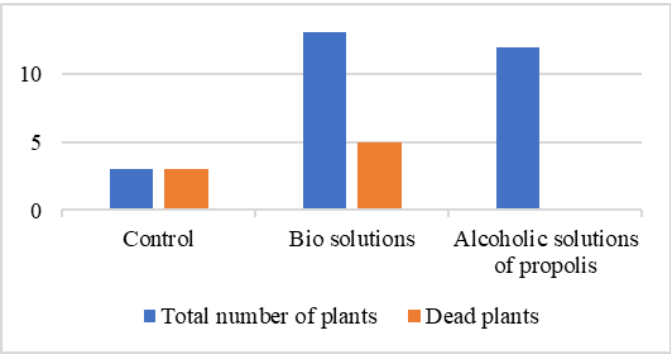
Only 4 days after on the 12th of August 2 more plants were infected. At the end of August,the number of infected plants has risen from 4 to all 15 plants, that kept growing fruits, graph 1. The first fruits occurred on the 13th of July and in that period PIS „Vojvodina“ recommended 5 treatments. By the end of the overall experiment, 5 plants have died from infection with *Phytophthora infestans* in a group of 13 plants.The third group of plants which consisted of 12 plants treated with alcoholic extracts of propolis dividedinto three groups of four depending on which concentration of the solution was used on them. From April until August, there were non of the symptoms shown on plants. The experiment stopped on 30th August, ten days after the first symptoms were noticed on plants treated with an alcoholic solution of propolis 0,10%, and they occurred as depigmentation on the bottom leaves, and non of the fruits were infected.

Table 3. Amount of precipitation during the study period

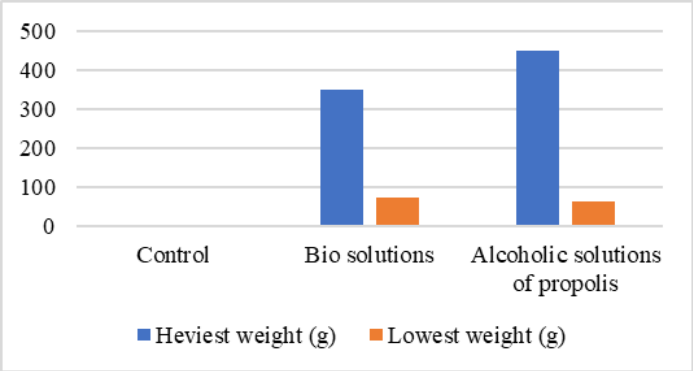
Date	Amount of Precipitation (mm)	Date	Amount of Precipitation (mm)
6th April	10	5th July	3
7th April	35	26th July	38
10th April	40	9th August	22
17th April	5	12th August	20
28th April	60	13th August	28
31th April	5	14th August	32
8th March	30	20th August	22
10th March	5	24th August	40
11th March	3	29th August	35
17th June	20	30th August	24
Total			**20**

Table 4. Treatments provided based on the recommendation of PIS „Vojvodina“

TR Treatment	Total Number of Treatments	Date	Karenca
		24th June	
Milk+baking soda		27th June	/
Detergent+cinnamon	12	29th June	/
Nettle solution		6th July	/
		10th July	
		11th July	
		13th July	
Alcoholic solution of propolis conc. 0,10%	12	29th July	/
Alcoholic solution of propolis conc. 0,15%	12	8th August	/
Alcoholic solution of propolis conc. 0,20%	12	15th August	/
		20th August	
		25th August	/
Total	**12**		***/***



Graph 1. Number of plants that have died of infection



Graph 2. Comparison of weights for all 3 groups

Table 5. Average weight

Experiment	Average Weight Per Plant (g)
Control	0
Bio extracts	2580
Alcoholic extracts of propolis	4920

When comparing the amount of given fruit produced by plants in different groups difference is small. Obviously, there was no way to measure or weigh the amount of produced fruit in a control group because all plants have died, in conclusion, production was 0 kg/plant. A group of 13 plants had their fruit production a little bit lower, 5 plants by the end of the experiment died. The smallest and easiest fruit was with a weight of 73,25g and the heaviest fruit was 350,14g.The average amount of fruit for this group was 2,58kg/plant. In a group of 12 plants were noticed larger fruits whose weight ranged from 150,53g to 450,75g, graph 2. The smallest weight in this group was just 62,54g. The average weight of fruit for this group was 4,92kg/plant. The average weight of the yield per plant treated with organic preparations was 2580g, and the one treated with an alcoholic solution of propolis was 4920g., Table 5.

4. CONCLUSIONS

1. On plants that have not been treated in any way, it has been proven how invasive *Phytophthora infestans* itself is. Young plants died very quickly, before the flowering period itself, so it was not possible to measure the average weight of the fruit.
2. When comparing the effectiveness of the preventive effect of alcohol solutions of propolis with other biological preparations, a better and stronger effect is clearly observed, without harmful effects on the environment.
3. When using alcoholic solutions of different concentrations, a wider range of effects was observed, not only on *Phytophtor infestans* but also on aphids, which reduces the total time spent on making other preparations that are allowed in organic agricultural production, but also increases economic profitability.
4. If we take into account that the control that was not treated at all was used in the other two trials, the conclusion is drawn that the results of the overall research are satisfactory, because the plants were exposed to negative environmental influences during the entire vegetation period.
5. When conducting research for the prevention and occurrence of various fungal diseases on plants, propolis showed a wide spectrum of action in different concentrations of the alcoholic extract, which was confirmed by various researches.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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