APPLICATION OF PROPOLIS SOLUTIONS IN PREVENTION OF THE OCCURRENCE AND DEVELOPMENT OF *Phytophthora infestans* IN ECOLOGICAL AGRICULTURAL PRODUCTION OF TOMATOES (*Solanum lycopersicum)*

Abstract

Organic Agricultural Production is a special system of food production that exists in a rules-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 impacting local ecosystems throughout the growth cycle.The use of phytopharmaceutical supplements 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 supplements with pesticidal and antifungal properties.

The aim of this paper is to investigate the effects of propolis solutions on the growth and development of Phytopathological fungi, presenting an alternative to other phytopharmaceutical supplements.

In this paper, the "American" tomato species are used throughout as it represents a common supermarket foodstuff. The following are the conclusions central to this paper: (1) preventative usage of alcoholic solutions impacts the likelihood of development of *Phytophthora infestans* and its subsequent growth while having a reduced environmental impact (2) a comparison of propolis solutions with other staple organic solutions 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 solutions, however, a combined alcoholic propolis solution was found to have further benefits, specifically in the prevention and neutralization of aphids.

Key words: propolis, phytopathological fungus, prevention, organic agriculture, tomato, *phytophthora infestans*

Introduction:

Organic agricultural production represents a special system of plant growth and harvest. It employs highly specific ecological practices tailored to 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 typical 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 effective 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 propolis in alcohol solutions. 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

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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 solutions for fungi will enable future generations to survive.

Tomato is a herbaceous plant from the family of *Solanaceae*, figure 1. Tomatoes are native to Central and South America, from which it was introduced across Europe by the Spanish in the 16th century. Its popularity drove increased production which has resulted in a regional monoculture across parts of Spain, Greece, the Netherlands, and Italy with the latter producing 7MMT (million metric tonnes) per annum. 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.

Picture number 1. Tomato fruit treated with an alcoholic solution of propolis conc. 15%

The use of alternative control methods that are efficient and have been proven on a regular basis, have a low potential for environmental contamination, meaning they are safe for the local ecosystem, and do not cause damage to the health of the applicator have been the subject of various studies, such as those conducted by Bettiol *et al.* (1999), Carneiro *et al.* (2007) and Faria *et al.* (2011), among others. 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 commondisease 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. The first symptomsoccur on leaves positioned lower on a stem, they develop spots that spread and cause leaves to go black, which is a sign of infection. *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 withoutdiseases, 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.

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Agriculture was developed to produce crops and livestock for human consumption. 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 arevery 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 able to be broken down. Once they enter the body of an organism, they are permanently stored in the body tissue [12].
PathogenPhytophthora *infestans* is a polyphagous fungus. It can infect potatoes, aubergine, tomatoes, and many more plants. Symptomsoccur on leaves, stems, and fruit. The firstsymptoms can be noticed in any micro-location on a plant where the best conditions for development are located. The most common place for first symptomsisthe oldest leaves that are positioned lowest on the stem. The color and size of depigmentationdepend 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. It can variate 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 represent a group of polyphenol compoundsthat can be isolated from plants. The main structure of flavonoids contains two benzene nuclei connected with a chain of 3 carbons located on the second, third, or fourth atom on the chromatic ring. 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].

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 solutions were made with propolis collected from *Apis mellifera* – a European honey bee from the city named Gunja in Croatia. Propolis solutions were prepared in 3 different concentrations according to the recommendation of Tringale[14]. table of contents number 1.

Table of contents number 1. Recommendation of Tringale[16], for usage of solutions

Fruit	Disease or pest	Usage of the propolis solutions
Actinidija	Gray mold (*Botrytis)*	0,2% hydro-alcoholic solution + 0,3% wettable sulfur
	Aphids	2-3 treatments with the alcohol solution
Citrus fruits	Anthracnose (*Colletotrichum gloesporoides*)	After removing the infested branches carry out 2-3 treatments with 0,2% hydro-alcoholic solution
	Fruit mold (*Phytophtora citrophtora*)	Treat fruits before or immediately after harvest with 0,1% alcohol solution and allow them to air dry
	Thyroid aphids	Coat the infested branches with propolis oil

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	(*Mytiloccocus beckii, Lepidosaphes gloverii* i dr.)	
Olive	Olive fly (*Dacus oleae*)	When the attack is not stopped in time, an alcoholic solution of 0,1% + wettable sulfur effectively destroys the eggs of the olive fly inside the fruit
	Cancer (*Pseudomonas savastanoi*)	2-3 treatments with 0,2% hydro-alcoholic solution
	Thyroid aphids (*Lepidosaphes destefani, Lucaspis riccae*)	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%)
	Gray mold (*Botrytis cinerea*)	Carry out treatments with hydro-alcoholic solution (0,2%) + wettable sulfur (0,2-0,3%) according to meteorological conditions

This experiment included the usage of 0,20%, 0,15%, and 0,10% concentrations. They have been carefully prepared in a couple of steps. The first step was freezing propolis and then grounding 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 solutions as previously stated were prepared in three different concentrations, which were stored in conditions without any sunlight and in dark glass bottles. Solutions were stored for 20 days and on the last day they were filtered.

Impact of alcoholic solution 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 28of which 12 of them were treated with alcoholic solutions of propolis, 15 plants were treated with other solutions 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 of contents number 2.

Table of contents number 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 solution	4
Tomato	S	Treated with a solution of milk + baking soda, nettle solution, detergent + cinnamon	13
Tomato	K	Not treated	3
Total			28

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

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 of contents number 3.

Table of contents number 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**

In the period of April until August, all three alcoholic solutions 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 on them non of 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 of contents 4.

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

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	

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		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		/

Propolis solutions 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 solutions and a mixture of detergent and cinnamon were used on a part of the plantation where alcoholic solutions of propolis were not used. This way was proven that propolis solutions have repellentproperties on *Aphidae*, they never occurred on plants treated with alcoholic solutions 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 solutions in order to compare the efficiency of alcoholic solutions of propolis with other solutions 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 weretreated with alcoholic solutions 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 number 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 solutions 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.

Graph number 1. Number of plants that have died of infection

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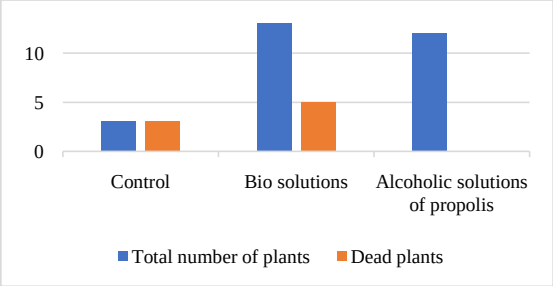

When comparing the amount of given fruit produced by plants in different groups difference is small. Obviously, there was no way to measure or weigh the amount of produced fruit in a control group because all plants have died, in conclusion, production was 0 kg/plant. A group of 13 plants had their fruit 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 number 2. The smallest weight in this group was just 62,54g. The average weight of fruit for this group was 4,92kg/plant, table of Contents number 5.

Graph number 2. Comparison of weights for all 3 groups

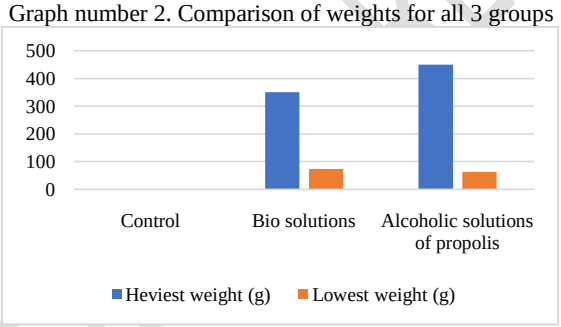

Table of contents number 5. Average weight

EXPERIMENT	AVERAGE WEIGHT PER PLANT (g)
Control	0
Bio solutions	2580
Alcoholic solutions of propolis	4920
Total	**9258**

Conclusions:

1. The best way of controlling pathogens to prevent their development is by applying all of the preventive measures, agrotechnical measures combined with alcoholic solutions of propolis, and other allowed solutions for organic agricultural production.
2. Possibilities of using propolis and its solutions in the ecological protection of plants are numerous, accent is given to the concentration which is used on plants to inhibit growth of phytopathological fungus.
3. The benefits of propolis solutions are enormous, the antifungal effect can be achieved on leaf curl, *Plasmopara viticola*, and *Phytophthora infestans*, but also in the fight

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against insects such as aphids, flies and they have a huge impact on improving the conditional state of a plant, stronger manifestation of plants vigor.

4. The use of propolis solutions in organic agricultural production can increase the sustainability of plant crops, given the fact that they are all-natural and not harmful to the environment.
5. Comparing the efficiency of alcoholic solutions of propolis and other bio-solutions, it is clear that solutions of propolis have a stronger effect on plants, without harmful effects on the environment.
6. Whider specter of efficiency is proven by having an indirectimpact in the prevention of the occurrence of aphids, without having a negative effect on them or any other useful insect. Simply solutions of propolis for an invisible coat over leaves and other parts of plants which disables insects to penetrate through it to the inside of the plant.

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