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RESEARCH ARTICLE

IMPACT OF PROPOLIS ON THE OCCURRENCE AND DEVELOPMENT OF PHYTOPATHOGENIC FUNGI

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Abstract

In every country around the world, phytopathogenic fungi cause enormous economic damage. With the growth of the human population, the agricultural production has significantly increased in monoculture, which caused enlarged development of phytopathogenic fungi on crops, orchard et cetera. Over time phytopathogenic fungi developed resistance on standard agricultural agents. Because of this, it is necessary to find new ways in battle against these kinds of fungi. The experimental part of this paper covers usage of propolis solutions in battle against of the occurrence and development of phytopathogenic fungi *Plasmopara viticola* and *Taphrinadeformans* in in vivo conditions on the grapevine (lat. *Vitis vinifera*) and peach (lat. *Prunus persica*). The aim of the study is to determine, with all necessary measurements like pruning, fertilizing, irrigation, time of application of the solution and weather forecast, possibility of usage propolis in influence on the occurrence and development of phytopathogenic fungi. In recent years, great efforts have been made to introduce natural disease control agents, such as propolis extract in alcohol and aqueous solution to reduce certain growth of phytopathogenic fungi in organic agriculture which can increase sustainable agricultural production.

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Introduction:-

In Bosnia and Herzegovina and in others states in the world, phytopathogenic fungi can cause really big economic damages in agricultural production. Today usage of fungicides is out of control, because they play a big part in fight against pests and diseases in agricultural production. In order to insure sustainable agricultural system, balance must be made between control risks of fungal diseases in crops and protecting aquatic and terrestrial ecosystems[1].

Over the last years, big efforts have been made to identify natural plant disease control products and possibility of usage natural compounds, such as propolis extracts in alcoholic solution in propose to reduce certain phytopathogenic fungi [2]. With global population growth, agricultural production has intensified and the number of producers in the organic production system has enlarged. In general, the term organic agriculture means a specific system of sustainable management in agriculture with the aim of producing healthy food, in order to meet the appropriate social and economic needs while preserving the natural ecosystem and landscape[3]. Fungal disease can damage plants and crops, causing major losses in agricultural activities and food production[4]. It is estimated that fungi are the highest threat for animal-host and plant-host species, representing the major cause (approximately

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65%) of pathogen-driven host loss. The general impact of fungal pathogens on human health goes beyond the ability of fungi to infect humans, since they destroy a third of all food crops annually causing economical loss and impacting global poverty. Statistics from the 2009–2010 world harvest [5] suggest fungi-induced losses in five of the most important crops globally (rice, wheat, maize, potatoes, and soybean). If those losses were mitigated, these crops would have been enough to feed 8.5% of the seven billion populations 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]. In this complex scenario, it is now clear that the global warming and accompanying climate changes have resulted in increased incidence of many fungal diseases [7]. Medically approved antifungal drugs have been used for agricultural purposes for decades. Emergence of antifungal resistance can endanger the already limited treatments options, with calamitous effects for treatment outcomes. The development of new antifungal drugs is urgent to improve both human health and agricultural production [8].

The use of alternative control methods that are efficient, have low potential for environmental contamination and do not cause damage to the health of the applicator have been the subject of various studies, such as those conducted by Bettiol, Carneiro and Faria, among others. Among the 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 *in vitro*. Due to its antimicrobial properties propolis has been utilised by man for millennia, however, only recently has its use as an agricultural antimicrobial agent been tested. The capacity of this product to activate plant defence mechanisms is highly promising, due to the large number of substances in its composition [10].

The ascomycetous fungus *Taphrina deformans* is the agent of peach leaf curl, a worldwide disease of peach potentially devastating to both crop yields and tree longevity. Conspicuous leaf curl symptoms result from the invasion of host tissue by the strictly parasitic mycelial phase of the *T. deformans* dimorphic life-cycle [11]. Peach leaf curl is one of the most common diseases of peach. Symptoms first appear in early spring on expanding foliage. Young, infected leaves become thickened and distorted along the midrib and take on a red to purplish hue. Later, as the fungus begins to produce spores. Diseased leaves eventually die and fall off the tree. These leaves are replaced by a second growth of foliage. Blossoms, young fruit, and succulent shoots also may be infected. Because infected blossoms and fruit crop rapidly, symptoms on these parts may be more difficult to observe. Extensive defoliation can lead to a reduced fruit crop the following year. In addition, defoliation weakens trees and predisposes them to other diseases and to winter injury. Peach leaf curl can be controlled effectively with application of appropriate fungicide [12]. The problem arises because the fungicide needs to be applied several times during the year to prevent the occurrence and further spread of *T. deformans*. These types of fungicides are not allowed in organic farming, so according to the recommendation of Tringale [13], it is possible to use an alcoholic solution of propolis in the fight against curling of peach leaves.

Downy mildew is induced by *Plasmopara viticola*. The term “mildew” refers to the cottony white growth that develops on infected tissue under moist conditions. The spores, called sporangia, germinate to produce several flagellated zoospores. Zoospores possess a short motile stage, during which they swim toward the stomata. After adhering to the plant surface, the spore begins to penetrate the host. Consequently, downy mildew is a serious problem only under conditions in which rainfall is prevalent throughout much of the growing season. They ramify extensively throughout host tissues. Under moist conditions, sporulation develops rapidly. During the summer, the fungus produces a resting stage called an oospore. Oospores may remain dormant for several years, until conditions favorable for germination result in spore production. Typically, this occurs in the spring. Newer systemic fungicides, such as fosetyl aluminum and phenylamides, are especially effective due to their incorporation into plant tissues. The uptake of the fungicide by plant tissues also reduces their removal by rain [14]. To solve the problem of removal of fungicides by rain and to use less fungicides, according to the recommendation of Tringale [13], it is possible to use hydro-alcoholic solution of propolis which is approved to use in organic agricultural production.

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 the 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 mobile in the

environment and often move through water, air and soil. The problem with pesticide mobility is that when they travel, the pesticides come in contact with other organisms and can cause harm. Pesticides have also been shown to disrupt the balance of an 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. In addition to causing harm to wildlife, pesticides that travel from their original location are known to cause harm to humans. 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[15].

Materials and Methods:-

The experiment was conducted at Family Agricultural Household „Atiković“ in Tuzla, at 508 metres altitude, from March 2020 to August 2020 for *Plasmopara viticola* and from March 2020 to May 2020 for *Taphrina deformans*.

The propolis used in the experiment was collected from *Apis mellifera* – European honey bee from the city Gunja in Croatia. Propolis solutions were prepared in 3 different concentrations according to the recommendation of Tringale[13], table of content number 1.

Table 1:- Recommendation from Tringale ⁽¹⁴⁾, for usage of the solutions.

Fruit	Disease or pest	Usage of the propolis solutions
Actinidija	Gray mold (<i>Botrytis</i>)	0,2% hydro-alcoholic solution + 0,3% wettable sulfur
	Aphids	2-3 treatments with alcohol solution
Citrus fruits	Anthracnose (<i>Colletotrichum gloeosporoides</i>)	After removing the infested branches carry out 2-3 treatments with 0,2% hydro-alcoholic solution
	Fruit mold (<i>Phytophthora citrophthora</i>)	Treat fruits before or immediately after harvest with 0,1% alcohol solution and allow them to air dry
	Thyroid aphids (<i>Mytilococcus beckii</i> , <i>Lepidosaphes gloverii</i> i dr.)	Coat the infested branches with propolis oil
Olive	Olive fly (<i>Dacus oleae</i>)	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 (<i>Pseudomonas savastanoi</i>)	2-3 treatments with 0,2% hydro-alcoholic solution
	Thyroid aphids (<i>Lepidosaphes destefani</i> , <i>Lucaspis riccae</i>)	Coat the infested branches with propolis oil
Peach	Curliness (<i>Taphrina deformans</i>)	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 (<i>Plasmopara viticola</i>)	According to meteorological conditions, carry out numerous treatments with hydro-alcoholic solution (0,2%) + wettable sulfur (0,3%)
	Gray mold (<i>Botrytis cinerea</i>)	Carry out treatments with hydro-alcoholic solution (0,2%) + wettable sulfur (0,2-0,3%) according to meteorological conditions

First step was to freeze the propolis. Frozen propolis was ground to a fine powder and mixed with water or 97% denatured alcohol. In every mixture was added 1g of soy lecithin and an appropriate amount of pure sulfur. Aqueous solution of propolis was made in 3 different concentrations: 0,20%, 0,15% and 0,10%. Alcoholic solution also was made in 3 different concentrations: 0,20%, 0,15% and 0,10%. Also hydroalcoholic solutions were made in three different concentrations: 0,20%, 0,15% and 0,10%. The mixtures were stored in dark glass bottles, which were closed and kept at the room temperature in a secure place. Aqueous solution was stored and mixed daily for 14 days, alcoholic solution was stored for 20 days and the last day solutions were filtered.

Impact of alcoholic solution of propolis on Taphrinadeformans:

The experiment was conducted in in vivo conditions in environment which was not free from the disease pathogen. For experiment were used 4 trees of peach *Prunuspersica*. One tree was used as a control and other three trees were sprayed with an aqueous solution of propolis. Every tree had a mark for the appropriate concentration of the solution by which it was sprayed.

Application of the treatments was performed manually using a sprayer with a conical nozzle until the leaves were completely wet. All four trees were previously infested with *Taphrinadeformans*. Treatments were performed according to recommendation of "PIS VOJVODINA" from: 2nd March, 2020 to 2nd May, 2020, table of content number 2.

Table 2:- Time period of performing the experimental part on *Taphrinadeformans*.

Tree label	Time period
Tree number 1 - control	2 nd March – 3 rd April
Tree number 2 – 0,10%	2 nd March – 2 nd May
Tree number 3 – 0,15%	2 nd March – 2 nd May
Tree number 4 – 0,20%	2 nd March – 2 nd May

Results and Discussion:-

During the entire period of monitoring whether the occurrence of *Taphrinadeformans* will occur on peach trees, agro-technical measures were performed: pruning, fertilization, irrigation, cultivation of row and inter-row space. Special attention is paid to the fact that the cause of peach leaf curl can not be subsequently controlled and that protection must be carried out exclusively preventively.

In the period from March to May, two concentrations of alcoholic propolis solutions proved to be successful. No symptoms appeared on trees treated with an alcoholic solution of propolis at a concentration of 0,20% and 0,15%, but on the control tree and a tree treated with an alcoholic solution of propolis 0,10% there occurred symptoms such as curled leaves, which were bubbly and wrinkled, drying of shoots and eventually decay, drying of the whole plant. At the control, which was the first tree in March (16th March, 2020), the leaves curled and the tree dried up at the beginning of April. A tree treated with an alcoholic solution of propolis 0,10% first symptoms were observed in April (8th April, 2020), the plant continued to develop further, but there was no fruiting, table of content number 3.

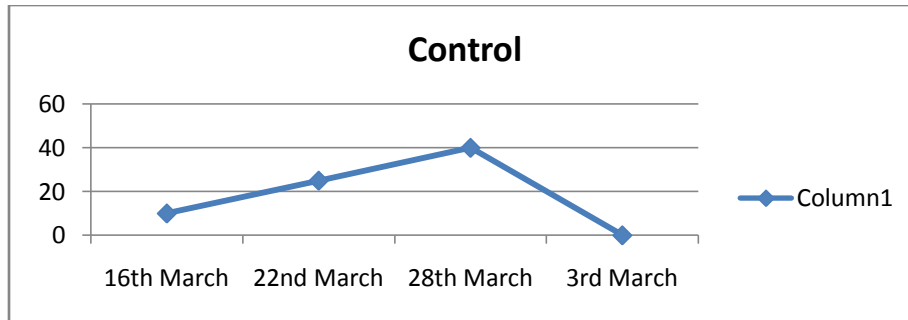
Table 3:- Monitoring the appearance of the first symptoms on the control tree and tree number 2.

Tree label	Date of onset of the	Number of diseased	Number of diseased
Tree number 1 – control	16 th March	10	1
Tree number 2 – 0,10%	8 th April	6	3

The trees were carefully inspected every third day. Treatments were performed depending on the weather, early in the morning or early evening, table of content number 4 and table of content number 5, chart number 1 and chart number 2.

Table 4:- Monitoring the increase in the number of diseased leaves on control.

Date	Number of diseased flowers
16 th March	10
22 nd March	25
28 th March	40
3 rd March	the tree withered

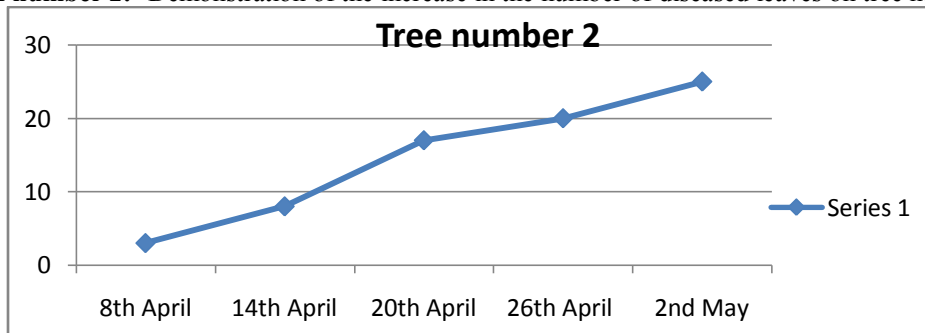


Graph number 1:- Demonstration of an increase in the number of diseased leaves on control.

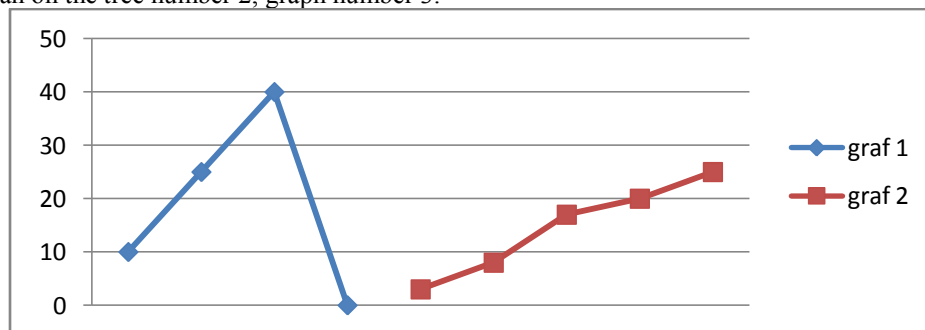
Table 5:- Monitoring the increase in the number of diseased leaves on tree number 2.

Date	Number of diseased flowers
8 th April	3
14 th April	8
20 th April	17
26 th April	20
2 nd May	25

Graph number 2:- Demonstration of the increase in the number of diseased leaves on tree number 2.



If we compare the control tree and the tree number 2, we can see that the disease developed more strongly on the control tree than on the tree number 2, graph number 3.



Graph number 3:- Comparison of increase in number of diseased leaves between control and tree number 2.

In this study 0,20% and 0,15% alcoholic solutions showed a positive effect on inhibiting the occurrence, growth and development of Taphrinadeformans, picture number 1 and picture number 2.



Picture number 1:-
Peach tree treated with an alcoholic solution of propolis conc.0,15%.



Picture number 2:-
Peach tree treated with an alcoholic solution of propolis conc. 0,15%.
0,10% solution showed a negative effect on inhibiting the occurrence and growth and development of Taphrinadeformans picture number 3.



Picture number 3:-

Peach tree treated with alcoholic solution conc. 0,10%

Impact of hydroalcoholic solution of propolis on *Plasmoparaviticola*:

The experiment was conducted in in vivo conditions in environment which was not free from the disease pathogen. For experiment were used 4 rows of grapevine type Strašenski. One row was used as a control and other three rows were sprayed with an hydroalcoholic solution of propolis. Every row had a mark for the appropriate concentration of the solution by which it was sprayed.

Application of the treatments was performed manually using a sprayer with a conical nozzle until the leaves were completely wet. All four rows were previously infested with *Plasmoparaviticola*. Treatments were performed according to recommendation of "PIS VOJVODINA" from: 9th March, 2020 to 1st August, 2020., table of content number 6.

Order label	Time period
Order number 1 – control	9 th March – 1 st August
Order number 2 – 0,10%	9 th March – 1 st August
Order number 3 – 0,15%	9 th March – 1 st August
Order number 4 – 0,20%	9 th March – 1 st August

Results and Discussion:-

In the period from March to August, all three concentrations of hydroalcoholic solution of propolis proved to be successful. There were no symptoms, such as "oil stains" on the leaf, white coating on the berries, white coating on the shoots and no drying of the flowers. At the control, that is, on the first row, some "oil stains" appeared on some vines in June (15th June, 2020), but those leaves were removed and burned, and no further symptoms appeared, table of content number 7.

Table 7:- Monitoring of the appearance for first symptoms.

Order label	Date of onset of first symptoms	Number of diseased leaves
Order number 1 – control	15 th June	20
Order number 2 – 0,10%	/	/
Order number 3 – 0,15%	/	/
Order number 4 – 0,20%	/	/

The vineyard is carefully inspected every third day. Treatments were performed depending on the weather, early in the morning or early evening.

In this study 0,20%, 0,15% and 0,10% hydroalcoholic solutions showed a positive effect on inhibiting the appearance, growth and development of *Plasmopara viticola*, picture number 4, picture number 5 and picture number 6.



Picture number 4:-
Grapevine treated with a hydro-alcoholic solution of propolis conc.0,10%.



Picture number 5:-
Grapevine treated with a hydro-alcoholic solution of propolis conc.0,15%



Picture number 6:-
Grapevine treated with a hydro-alcoholic solution of propolis conc.0,20%

Conclusions:-

Control of these pathogens is carried using agrotechnical and chemical control measures, which is more economically accepted but it is also unacceptable for the environment, animals and humans. An alternative to control these pathogens is an ecological method of control which includes propolis.

The possibilities of using propolis in the ecological protection of plants are numerous. The most significant use of propolis is in the preparation of solutions of different concentrations in the inhibited growth of phytopathogenic fungi on plants.

The benefits of propolis solutions are numerous, where the antifungal effect on gray mold, anthracnose, leaf curl, downy mildew, but also insecticidal action in the fight against aphids, aphids, olive flies, in addition improves the condition of the plant, stronger manifestation of plants vigor and plants are more resistant to external negative factors of external conditions.

The use of propolis solutions in organic agricultural production can increase sustainability of plant crops, given the fact that they are all natural and not harmful for the environment.

These kinds of solutions are expensive because we do not have enough beekeepers in Bosnia and Herzegovina. Recommendation for every agriculturist is to put on 1 dunum of land 2 – 3 hives, which would enable additional income, better pollination and available propolis for preparation of solutions.

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