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EFFICACY OF PLANT EXTRACTS APPLICATION ON SUPPRESSION OF BOTRYTIS CINEREA AND IMPACT ON BLACKBERRIES YIELD

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Summary: Field tests of efficacy of nettle, dandelion and alfalfa water extracts application were conducted on organic plantation. Laboratory tests are performed at the Agricultural Institute of Republic of Srpska - Banja Luka and Veterinary Institute "Vaso Butozan" in Banja Luka. It was analyzed the following properties of water extracts: health safety, efficacy in preventing the development of *Botrytis cinerea* in, in vitro conditions" and impact on the yield of blackberries. Control of the microbiological safety of the water extracts had shown that they were health safety and can be used for foliar treatment of blackberry bushes. It was found that extracts of nettle with fermentation time of 14 and 21 days showed medium inhibitory effect on mycelium growth of the fungus *Botrytis cinerea*. There was no inhibitory effect of dandelion and alfalfa extracts on mycelium growth of the fungus *Botrytis cinerea*. All tested treatments achieved a higher yield of blackberries compared to the control. The highest average yield of blackberry was achieved by treatment with water extract of alfalfa. Fermentation time did not affect the yield of blackberries in any of the treatments.

Key words: plant extracts, nettle, dandelion, alfalfa, *Botrytis cinerea*, yield, blackberries

INTRODUCTION

Production of healthy safe food without use of pesticides, while preserving biodiversity and biological activity of the soil, is the main goal of organic agriculture. When using this method producer must eliminate the application of mineral fertilizers in production cycle. In these circumstances, in order to avoid reduction in yield, there is a need to replace mineral fertilizers with more organic fertilizers. Among the organic fertilizers, foliar fertilizers are particularly important, due to possibility to use during the whole growing season. When using organic fertilizers producer should be careful, because their untimely and excessive use, due to intense mineralization, may cause some negative consequences such as a significant increase of mineral forms of nitrogen in the soil. Because of the negative consequences that may arise during the application of organic fertilizers in health food production, more popular is the use of foliar organic fertilizers, because they also provide healthy products and preserve the environment.

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A large number of plant species that could be used for the production of high quality foliar plant solution are growing in nature. Prepared extracts of these plants are a source of readily available nutrient elements for growing crops. Concoctions, in addition to providing a sufficient amount of nutrients for plants, also have partially insecticidal and fungicidal effects due to bioactive chemicals that are found in solution (Kim et al., 2005; Daoubi et al., 2005).

In various aqueous extracts of herbaceous plants the presence of antibacterial, antifungal and insecticidal effects were found during laboratory testing (Satish et al., 1999; Bouamama et al., 2006; Ergen et al., 2006; Kiran and Raveesha, 2006; Okigbo and Ogbonnaya, 2006).

The application of water extracts made from different plants provides an opportunity for producers to be able to count on the good yield and on the possibility of easy selling. At the same time, the use of organic foliar fertilizer has no negative impact on the environment. Studies have shown that foliar fertilization in some cases may be 8 to 10 times more effective than the taking of nutrients from the soil, and that about 90% of the nutritional solution can be found in the lowest parts of the roots of the plant 60 minutes after application (Akanbo et al. 2007). An important characteristic of foliar fertilizers is that they directly absorbed into the plant and in limited quantities, unlike fertilizers that are given through the soil (Fernandez and Eichert, 2009).

Use of organic foliar fertilizer essentially supports the concept of sustainable agriculture. Bearing in mind that the water extracts are environmentally friendly, research should make a significant contribution to the advancement of organic fruit in our country, and beyond. Plant extracts are products which can be a significant source of various trace elements, depending on the type and quality of the land on which the plants were grown (Popescu et al., 2010).

The aim of this study was to carry out health control of the water solutions, examine their inhibitory effects on *Botrytis cinerea* that causes Fruit Rot in the "in vitro" conditions and determine the effect on the yield of blackberries.

MATERIALS AND METHODS

In 2010 and 2011. tests of health safety, chemical composition, the inhibitory effect of water extracts of nettle, dandelion and alfalfa on the *Botrytis cinerea* that causes Fruit Rot in the "in vitro" conditions and the influence of the extracts on the yield of berries, was conducted at the experimental field in Kozarska Dubica and in laboratories of the Agricultural Institute of the Republic of Srpska and Veterinary Institute "Vaso Butozan" in Banja Luka.

Two-factorial study (factor A – plant extract, factor B - the number of fermentation days) in four replications in a randomized block design, was set up in 2010, at an organic plantation in the village of Brekinja, Kozarska Dubica. Each replication consisted of five blackberry bushes.

For this study we it was used water extracts of nettle (*Urtica dioica* L.), dandelion (*Taraxacum officinale* F. H. Wigg.) and alfalfa (*Medicago sativa* L.), with periods of fermentation 14 or 21 days. Studies were included in the following treatments aqueous extracts:

1. The water extract of nettle with 14 days of fermentation
2. The water extract of nettle with 21 days of fermentation
3. The water extract of dandelion with 14 days of fermentation
4. The water extract of dandelion with 21 days of fermentation
5. The water extract of alfalfa with 14 days of fermentation
6. The water extract of alfalfa with 21 days of fermentation
7. The control treatment (untreated plants).

The water extracts of the alfalfa, dandelion and nettle after completion of the fermentation cycle, was drained before the foliar application, and then diluted with rain water to a ratio of 1:10. Spraying was performed with engine sprayer WILAGER DM 25, the volume of 15 l. First treatment was on May 18, 2011. and treatments were performed every 10 to 15 days. Treatments were repeated until the first ripe fruits of blackberries.

Safety control of water extracts used in our research was conducted in the Veterinary Institute "Vaso Butozan" in Banja Luka. The extracts were tested for the presence of bacteria according to the following standards: *Salmonella* species in 25 g; Coagulase-positive *staphylococci* in 0.01 g / ml, sulfitereducing *clostridia* in 0.01 g / ml; *Preoteus* species of 0.001 g / ml of *Escherichia coli* in 0.001 g / ml.

Analysis of the chemical composition of the water extracts of nettle, alfalfa and dandelion were performed in the laboratory for Agrochemistry and Agroecology at the Agricultural Institute in Banja Luka. It was analyzed the following parameters.

Nitrogen: Kjeldahl method, wet combustion (conc. H₂ SO₄ + H₂O₂ + 450 °C - Kjeltex system I, Tecator), distillation.

Phosphorus: wet burning with acid mixture ($\text{HNO}_3 + \text{HClO}_4 + \text{H}_2\text{SO}_4$) - from the solution, the determination of phosphorus staining method (coloring in yellow) by reading the optical density at spektrofotometru.

Potassium: wet burning acid mixture ($\text{HNO}_3 + \text{HClO}_4 + \text{H}_2\text{SO}_4$) - from the solution, the reading of potassium by flame photometry.

A large number of samples of diseased fruits were collected from experimental blackberries plantation. Samples were analyzed in the phytopathology laboratory at Agricultural Institute of Republic Srpska - Banja Luka. It was isolated several pure cultures of the fungus *Botrytis cinerea* by using standard laboratory method. After that analysis of the inhibitory actions of the water extract on mycelial growth.

For laboratory testing of extracts isolate of the fungus isolated from decaying fruit blackberries with experimental plantings of Kozarska Dubice was used. Before the experiment started, a sterile filtration of the extract through the filter paper, MN GF-6 with the pore diameter of 0.6 μm , a thickness of 0.35 mm and a diameter of 45 mm \varnothing were performed, in order to remove the impurities and microorganisms, which may contaminate the test culture. During the experiment, Petri dishes 90 mm in diameter into which it was poured in a thin layer of potato-dextrose agar were used.

The experiment is set according to the revised method of Guttersonet *al.*, 1986., with metal cutter in potato-dextrose agar to make three clip diameters 0.5 cm in which a micropipette poured about 120 μl of filtered water extract of nettle, dandelion and alfalfa. Distance between clips and fragment of the colony is 3 cm.

With the help of label that sticks under the Petri dish - where four circles was outlined, three for positioning clips that are made in a prepared agar to be used to pour the tested extracts, and the central position which will set the clip colonies tested fungus. The experiment was set up in four repetitions, and the evaluation of the inhibitory action of water extracts on the development of *Botrytis cinerea* colonies. It was done for 7 days after the development in a thermostat at a temperature of 25°C. Determining the efficiency of the tested extracts was determined by measuring the inhibition zone around the clip to extract.

The first harvest of ripe blackberry fruit was on 14 July, at the stage of their physiological maturity. By the end of August blackberry was harvested six times. During harvest, the yield measurement was performed for each blueberries bush using the precise scale „TECHNIQUE". By measuring the weight of harvested fruits we get the data on the yield of all the variants, including the control.

The research results collected were statistically analyzed using ANOVA, and significant difference between the mean values was determined by *LSD-test*.

RESULTS

Health safety of water extracts - Before the treatment blackberries with water extracts presence of harmful bacteria was tested. Results are shown in table 1.

Table 1. Presence of harmful bacteria in the water extracts of nettle, dandelion and alfalfa

Bacteria	Extract					
	14 days of fermentation			21 days fermentation		
	Nettle	Dandelion	Alfalfa	Nettle	Dandelion	Alfalfa
<i>Salmonella</i>	neg.	neg.	neg.	neg.	neg.	neg.
<i>Staphylococcus</i>	neg.	neg.	neg.	neg.	neg.	neg.
<i>Clostridia</i>	neg.	neg.	neg.	neg.	neg.	neg.
<i>Preoteus</i>	neg.	neg.	neg.	neg.	neg.	neg.
<i>Escherichia coli</i>	neg.	neg.	neg.	neg.	neg.	neg.

Chemical composition of water extracts - To determine the content of macro elements in water extracts of nettle, dandelion and alfalfa chemical analysis was made (Table 2). Examination of the contents of macroelements in water extracts is important, because they directly affect the yield, quality and ability to keep the fruits. Macroelements are important, because they influence the resistance to plant diseases.

Table 2. The chemical composition of water extract of nettle, dandelion and alfalfa with fermentation period of 14 days

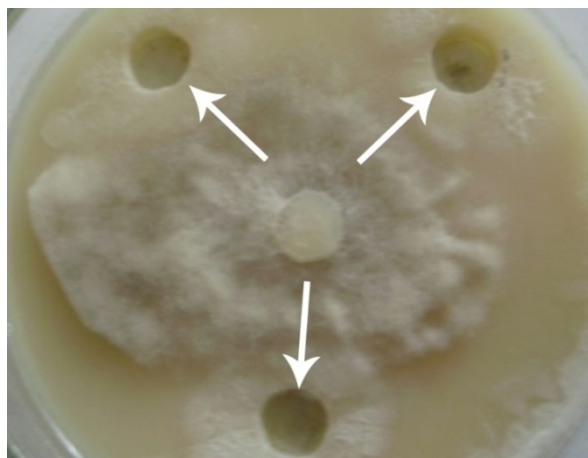
No.	Extract	pH	N (mg l ⁻¹)	P (mg l ⁻¹)	K (mg l ⁻¹)
1.	Nettle	6.4	56.8	56.3	700.2
2.	Dandelion	5.2	41.0	42.5	1 205.3
3.	Alfalfa	5.4	105.6	40.2	670.4
LSD	0.05	0.31	3.05	2.07	68.55
	0.01	0.48	4.62	3.14	103.85

Study of inhibitory activities of water extracts on *Botrytis cinerea* in „in vitro conditions“- Large number of samples of diseased fruits of blackberries from which was isolated the fungus *Botrytis cinerea* by multiple pure cultures. After isolation inhibitory activity of water extracts on isolates was analyzed (Table 3).

Table 3. Results of the inhibitory effect of water extracts on the mycelia growth of *Botrytis cinerea*

Extracts	Isolate	
	17 days of fermentation	21 days fermentation,
Nettle	+++	+++
Dandelion	-	-
Alfalfa	-	-

- Without the expressed inhibitory effect
- + Very weak inhibitory activity (inhibition zone width of 0.1 - 0.5 cm)
- ++ Weak inhibitory activity (inhibition zone width 0.6 – 1.0 cm)
- +++ Medium inhibitory activity (inhibition zone width from 1.1 to 1.5 cm)
- ++++ Strong inhibitory activity (inhibition zone width from 1.6 to 1.9 cm)
- +++++ Very strong inhibitory activity (inhibition zone width from 2.0 to 2.2 cm)

**Figure.1.** Inhibitory effect of water extract of nettle on mycelia growth of *Botrytis cinerea*

Yield of blackberry - blackberry yield depends on many factors, of which the most important are: varieties, growing systems and agro ecological conditions, and yields obtained during these tests are shown in table 4.

Table 4. The effect of different water extracts to average yield of blackberries

Fermentation time	Extract			
	Control	Nettle	Dandelion	Alfalfa
14 days	9.02	10.55	10.21	10.91
21 days	9.01	10.26	10.37	10.97
\bar{X}	9.02	10.41	10.29	10.94
Level	A		B	AB
LSD	0.05	1.52	1.07	2.13
	0.01	2.04	1.45	2.88

DISCUSSION

Based on the results of control of microbiological safety of the water extract of nettle, dandelion and alfalfa with different number of days of fermentation, it can be concluded that it is satisfactory. In water extracts of the test results were negative for the presence of the following harmful bacteria: *Salmonella*, *Staphylococcus*, *Clostridia*, *Preoteus* species and *Escherichia coli*.

The results of these tests confirm that the extract was healthful and can be freely used for foliar treatment blackberries until the harvest.

Results of chemical analyzes of water extracts show that the pH of the extract of dandelion and alfalfa was acidic and nettle had weakly acidic reaction. Determined pH values ranged from 5.2 (dandelion) to 6.4 (nettle). The nitrogen content stands out in water extract of alfalfa (105.6 mg l^{-1}). The water extract of alfalfa had twice more nitrogen compared to the dandelion extract (41.0 mg l^{-1}). The highest content of phosphorus was found in the extract of nettle (56.3 mg l^{-1}), while the extract of dandelion had by far the highest content of potassium (1205.3 mg l^{-1}). Based on the results of chemical analyzes of water extracts, it was found that there were statistically significant differences in the pH value and the content of nitrogen, phosphorus and potassium between extracts of nettle, dandelion and alfalfa.

According to Verma and Dubey (1999) botanical pesticides, based on plant metabolites, certainly represent a quality alternative to synthetic pesticides because of their minimal impact on the environment and human health.

During research of water extracts, medium inhibitory effect on mycelial growth of *Botrytis cinerea* showed only extracts of nettle with 14 and 21 days of fermentation (Figure 1). Extracts of dandelion and alfalfa did not show any inhibitory activity *in vitro* on the tested pathogen.

These results are in compliance with the results of Bajpai et al. (2008) and Souleymane et al. (2010).

The results of these tests showed that the average yields of blackberries were from 9.02 t ha^{-1} (control) to 10.94 t ha^{-1} (alfalfa extract). In all treatments higher yield of blackberries achieved compared to the control. The highest average yield of blackberries was achieved with water extracts of alfalfa (10.94 t ha^{-1}). In these tests there was no significant difference in the yield of blackberry bushes in the treatment with water extracts which had different number of days of fermentation.

CONCLUSION

Based on the obtained results, the following can be concluded:

- Control of the microbiological safety of the water extracts of nettle, dandelion and alfalfa, showed that they were healthful and can be used for foliar treatment of blackberry bushes until the harvest.
- Chemical analyzes of water extracts showed that an extract of alfalfa contains mostly nitrogen, nettle extract phosphorus, dandelion extract potassium.
- Extracts of nettle with fermentation time of 14 and 21 days had a medium inhibitory effect on mycelial growth of the fungus *Botrytis cinerea*.
- All tested treatments achieved a higher yield of berries compared to the control. The highest average yield was obtained by treating the blackberry bushes with water extract of alfalfa. Fermentation time did not affect the yield of blackberries in any of the treatments.

Bearing in mind the increasing interest in natural control of plant pathogens and obvious increase demand for healthy safe food, as well as the fact that in our country is not much done on this issue, we find it necessary to continue testing in order to obtain more data on the use of water extracts.

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**ISPITIVANJE EFIKASNOSTI PRIMENE VODENIH EKSTRAKATA NA SUZBIJANJE RAZVOJA
BOTRYTISCINEREA I UTICAJA ISTIH NA PRINOS KUPINE**

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Izvod: Ispitivanje efekata vodenih ekstrakata koprive, maslačka i lucerke izvedeno je u zasadu kupine organske proizvodnje. Laboratorijska ispitivanja su izvedena u Poljoprivrednom institutu Republike Srpske – Banja Luka i Veterinarskom Institutu „Vaso Butozan“ u Banjaluci. Tokom istraživanja analizirana su sledeća svojstva vodenih ekstrakata: zdravstvena ispravnost, efikasnost u sprečavanju razvoja *Botrytis cinerea* u „*in vitro*“ uslovima i uticaj na prinos kupine. Kontrolom mikrobiološke ispravnosti vodenih ekstrakata, utvrđeno je da su oni zdravstveno ispravni i da se mogu koristiti za folijarno tretiranje žbunova kupine. Laboratorijskim ispitivanjima utvrđeno je da su ekstrakti od koprive sa periodom fermentacije od 14 i od 21 dan ispoljili srednje inhibitorno delovanje na porast micelije gljive *Botrytis cinerea*. Nije utvrđeno inhibitorno dejstvo ekstrakata maslačka i lucerke na porast micelije gljive *Botrytis cinerea*. Svi ispitivani tretmani ostvarili su veći prinos kupine u odnosu na kontrolnu varijantu. Najveći prosečan prinos kupine ostvaren je tretiranjem žbunova sa vodenim ekstraktom lucerke. Trajanje fermentacije nije uticalo na prinos kupine ni kod jednog tretmana.

Ključne reči: biljni ekstrakti, kopriva, maslačak, lucerka, *Botrytis cinerea*, prinos, kupina

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