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APPLICATION OF FERMENTED EXTRACT OF HORSETAIL (*Equisetum arvense* L.) TO CONTROL TOMATO LATE BLIGHT (*Phytophthora infestans* (Mont.) de Bary)

Vladimir Filipović [65]; Vera Popović [66]; Snežana Dimitrijević[67]; Vladan Ugrenović [68]; Sara Mikić[69]; Snežana Mrđan[70]; Ljubica Šarčević-Todosijević [71]*

Abstract

The paper examined the influence of the application of fermented extract of horsetail (*Equisetum arvense* L.) in order to control tomato late blight (*Phytophthora infestans* (Mont.) de Bary). Given that the European Union intends to reduce the total use of chemical pesticides of 50% by 2030., for this reason we need an alternative in the protection of plants and soil. The aim of the work arose from the need for a solution to one of the most economically significant diseases of tomatoes in our region. The location of the research was the collection of the Institute for the Study of Medicinal Plants "Dr. Josif Pančić" located in Pančevo (44°52'20"N; 20°42'06"E; 74 m.a.s.l.). The experiments were carried out during the growing season in 2020 with the tomato variety „Ox Heart" („Volovsko srce"), on the humogley soil type, according to a random block system in four replications.
After the evaluation of the infection of the plants by late blight, the best results were shown by the fourth variant (six weeks after the first treatment, i.e. four treatments of plants with fermented extract of horsetail) which had the least pathogen presence (on average 1.6 i.p.), while the worst results were achieved by the control variant in which there was the highest presence of the examined disease (on average 3.7 i. p.). The highest yield of tomato fruit was recorded on plants in the third variant, i.e. after three treatments or four weeks after the first treatment with fermented extract of horsetail.

[65] Vladimir Filipović, PhD, Senior Research Associate, Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia, vfilipovic@mocbilja.rs;
[66] Vera Popović, PhD, Principal Research Fellow, Academician of IRASA, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia, vera.popovic@ifvcns.ns.ac.rs;
[67] Snežana Dimitrijević, PhD, Research Associate, Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia, sdimitrijevic@mocbilja.rs;
[68] Vladan Ugrenović, PhD, Senior Research Associate, Institute of Soil Science Belgrade, Teodora Drajzera 7, 11000 Belgrade, Serbia, +381 (0)64 881-44-12, vladan.ugrenovic@gmail.com;
[69] Sara Mikić, MSc, Research Assistant, + Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia, smikic@mocbilja.rs;
[70] Snežana Mrđan, MSc, Research Assistant, Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia,, smrdjan@mocbilja.rs,
[71] Ljubica Šarčević-Todosijević, MSc, Research Assistant, High Medical - Sanitary School of Professional Studies, Tošin bunar 7a, 11080 Belgrade (Zemun), Serbia, ljsarcevic@gmail.com

Key words: *Horsetail fermented extract, Equisetum arvense, tomato, tomato late blight, Phytophthora infestans.*

Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop in the Republic of Serbia. It is a rich source of vitamins, minerals, and fiber, and a dietary source of antioxidants. Tomato late blight (*Phytophthora infestans* (Mont.) de Bary) is one of the most economically damaging diseases not only for tomatoes, but also for potatoes and eggplants. Tomato plants can be infected already at the seedling stage. Symptoms of the presence of this disease most often appear in the period from May to September, but since its development depends on temperature and humidity, in certain years, with insufficient rainfall and severe drought, the appearance of the disease may be absent [1]. In order to control it, in the production practice of tomatoes so far, mostly synthetic preparations were used. In addition to this way of fighting, some conventional and organic producers made sure that tomato plants are not planted in the shade, that crop rotation guidelines are followed, that wild tomato and potato plants are destroyed, mulching the inter-row space, and similar.

Horsetail is one of the medicinal plant species whose use as a means of plant protection, among other things, in the tomato crop has been allowed by the European Commission [2]. According to the aforementioned Regulation, the entire above-ground part of the plant can be used to protect against early blight (*Alternaria solani*) and septoria blight (*Septoria lycopsersici*). For this purpose, macerate of horsetail is used, 1.2 kg of dried horsetail is immersed in 10 liters of boiling water and left to stand for 24 hours, after which it is filtered and used for this purpose at most twice in a 14-day interval. A number of researchers had satisfactory results when using macerate based on horsetail in the protection of tomato blight [3,7,10]. Although it is a sought-after medicinal plant, horsetail has not been cultivated. Horsetail can be found in abandoned places, in ditches, near roads and on barren, uncultivated arable lands and sometimes it occurs in moist meadows [3].

The aim of our research was to investigate the potential impact of a plant preparation - a fermented extract based on horsetail as a means of possible suppression of tomato late blight.

Material and methods

The following plant material was used in the research: tomato (*Lycopersicon lycopersicum* L.) variety Volovsko srce produced by "Superior" d.o.o. from Velika Plana. Research was conducted during 2020 in the greenhouse and experimental field of the Institute for Medicinal Plant Research "Dr Josif Pančić" located in Pančevo (44°52'20"N; 20°42'06"E; 74 m.a.s.l.).

At the end of February, seeds to produce seedlings were sown in the greenhouse without additional heating in the manner shown in the research work Filipovic et al. [4]. After three months, more precisely in the middle of May, developed seedlings

were transplanted in the field, when the seedlings reached the most optimal stage for reception. Planting was done manually at a distance between rows of 80 cm, and in a row 40 cm plant from plant. In the experimental field, basil was grown (*Ocimum basilicum* L.) as a pre-crop to all examined species.

The trial was based on a completely randomized block design with a base plot size of 6.0 m^2 (2.5 m x 2.4 m) in four replications.

As a means of protecting tomatoes from late blight, the fermented extract of horsetail was used in the experiments, which is prepared by soaking 3 kg of fresh plants in 10 liters of water and leaving it to ferment for twenty days. When the fermentation stops (no new foam is formed), the mass is strained and 30 liters of water is added to the resulting solution and the plants are watered with it. During the experiment, they were applied in four variants with the use of fermented extract of horsetail: Variant I - first treatment three days after planting, Variant II - two weeks after the first treatment, Variant III - four weeks after the first treatment and Variant IV - six week after the first treatment. The fifth variant was a control variant, i.e. a variant without application of the fermented extract of horsetail. During the last treatment, the infection of the plants was evaluated for the appearance of the cause of tomato late blight, *Phytophthora infestans* (Mont.) de Bary), on a scale of 1 to 5 index points. Where number 1 is the least sensitive variant and the maximum value on a scale of 5 index points represents the most sensitive variant to the occurrence of this disease. Disease scale from 1 to 5, where 1 = no infection, 2 = 1-25% of leaf area blighted, 3 = 26-50%, 4 = 51-75%, 5 = 76-100% of leaf area blighted [5 ,6]. The assessments were performed by randomly selecting 10 tomato plants within each experimental plot and providing a visual disease rating estimate [7]. The total yield of tomato fruit was determined for each experimental plot and expressed as g/plant.

Meteorological data during the experimental vegetation period were obtained from the meteorological station of the Institute ˝Tamiš˝ Pančevo (Table 1).

Table 1. Meteorological data for vegetation period in 2020

Year	Monts	V	VI	VII	VIII	Σ	ẍ
2020	**P**	52,2	144,8	82,8	39,4	629,4	-
2020	**T**	16,1	20,2	22,4	23,9	-	12,7
2004-2019	**P**	75,4	86,5	61,2	50,1	669,1	-
2004-2019	**T**	18,3	22,1	23,8	23,8	-	12,7

* P = Precipitation, (mm); T = Temperatures, (°C)

The experimentally obtained data were subjected to the two-way analysis of variance (ANOVA) using a statistical system [8] and significance ratings derived from F-test and LSD-test for a significance threshold of 5%.

Results and Discussion

The appearance of the causal agents of tomato late blight (*Phytophthora infestans* (Mont.) de Bary)

The results of the investigation of the impact of the application of different variants of treatment with horsetail fermented extract on the appearance of the pathogen of tomato late blight (*Phytophthora infestans* (Mont.) de Bary)) are shown in table 2.

Table 2. The Analysis of variance of evaluation of infestation of tomato late blight

Source	DF	Sum of squares	Mean squares	F Value	Prob>F
Model	4	13.333	3.33325	32.15354	3.38169E-7
Error	15	1.555	0.10367		
Total	19	14.888			

Computed against model Y=Mean(Y)

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
Control	4	0	3.7	0.36515	0.18257
Variant I	4	0	2.875	0.29861	0.1493
Variant II	4	0	2.025	0.3594	0.1797
Variant III	4	0	1.65	0.33166	0.16583
Variant IV	4	0	1.55	0.23805	0.11902

Contrast	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
Variant I Control	-0.825	0.22767	-3.62368	0.0025	0.05	1	-1.31027	-0.33973
	-1.675	0.22767	-7.35716	2.37807E-6	0.05	1	-2.16027	-1.18973
Variant II Control	-0.85	0.22767	-3.73348	0.002	0.05	1	-1.33527	-0.36473
	-2.05	0.22767	-9.00429	1.94956E-7	0.05	1	-2.53527	-1.56473
Variant II Variant I	-1.225	0.22767	-5.38061	7.64344E-5	0.05	1	-1.71027	-0.73973
	-0.375	0.22767	-1.64713	0.12032	0.05	0	-0.86027	0.11027
Variant III Control	-2.15	0.22767	-9.44352	1.05563E-7	0.05	1	-2.63527	-1.66473
	-1.325	0.22767	-5.81984	3.37578E-5	0.05	1	-1.81027	-0.83973
Variant III Variant I	-0.475	0.22767	-2.08636	0.05443	0.05	0	-0.96027	0.01027
	-0.1	0.22767	-0.43923	0.66675	0.05	0	-0.58527	0.38527

*Tretman / Fisher Test / Analysis of the differences between the categories with a confidence interval of 95%

The best results, i.e. the lowest intensity of tomato late blight infection, had the IV variant, i.e. the plants treated six weeks after the first treatment, in which four treatments were performed with the fermented extract of horsetail (at the beginning, after two weeks, four and six weeks after the first treatment). The evaluated plants had an average of 1.6 index points, i.e. the lowest sensitivity to the appearance of this economically important tomato disease. The highest occurrence of the disease was recorded in the first (control) variant, i.e. in the variant without the application of fermented extract of horsetail, where the presence of this pathogen had a value of 3.7 i. p., thus representing the most sensitive variant to the appearance of this economically important tomato disease. In the research of Trebbi et al. [7] examined the influence of horsetail macerate, whose influence was similar to the effectiveness of copper application in reducing symptoms of late blight on organic tomato leaf tissue. Due to their positive properties, preparations based on horsetail have been approved by the EC for use in organic production and as a possible replacement for copper preparations. According to this decision, horsetail application was approved based on a concentration of 0.2 kg/hL [9], which is considered a typical appropriate concentration in the field [10].

In the research work, Garmendia et al. [11] used a fermented extract of *Urtica dioica* and the *Equisetum hyemale* came from the "Ortiga Amiga" Spanish trading house. The recommended *Urtica* and *Equisetum* fermented extract dose (RD) was 10% v/v. The *Urtica* and *Equisetum hyemale* fermented extract chemical analyzes showed very low levels of nitrogen, phosphorus, and potassium. Unfortunately, due to poor mineral nutrition with the *Urtica* and *Equisetum hyemale* fermented extract, tomato late blight (*Phytophthora infestans* (Mont.) de Bary) was found on potato plants. Depending on modalities, *E. arvense* decoction seems to have a similar effect to "Full Copper" on fruits [12]. In addition to silicic acid, horsetail is rich in total phenols, total flavonoids and anthocyanins, and refractometric determination of soluble solids [13]. Preliminary field trials by Marangoni et al. [14] carried out in the area of Bologna (Italy) showed a satisfactory effect of foliar applied macerate based on horsetail, which reduced the percentage of cracking of sweet cherries fruits (cv. Van) to a similar extent as the application of preparations based on calcium chloride and silicon (sodium silicate).

Tomato fruit yield in plants treated with horsetail fermented extract

The results of the investigation of the impact of the application of different variants of treatment with horsetail fermented extract on the tomato fruit yield are shown in table 3.

Table 3. Analysis of variance of the effect of the application of horsetail fermented extract on the tomato fruit yield (g/plant)

Source	DF	Sum of squares	Mean squares	F Value	Prob>F
Model	4	1.22079E6	305196.81075	16.98774	1.92066E-5
Error	15	269485.595	17965.70633		
Total	19	1.49027E6			

Computed against model Y=Mean(Y)

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
Control	4	0	3.7	0.36515	0.18257
Variant I	4	0	2.875	0.29861	0.1493
Variant II	4	0	2.025	0.3594	0.1797
Variant III	4	0	1.65	0.33166	0.16583
Variant IV	4	0	1.55	0.23805	0.11902

Contrast	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
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Variant II Control	-0.85	0.22767	-3.73348	0.002	0.05	1	-1.33527	-0.36473
	-2.05	0.22767	-9.00429	1.94956E-7	0.05	1	-2.53527	-1.56473
Variant II Variant I	-1.225	0.22767	-5.38061	7.64344E-5	0.05	1	-1.71027	-0.73973
	-0.375	0.22767	-1.64713	0.12032	0.05	0	-0.86027	0.11027
Variant III Control	-2.15	0.22767	-9.44352	1.05563E-7	0.05	1	-2.63527	-1.66473
	-1.325	0.22767	-5.81984	3.37578E-5	0.05	1	-1.81027	-0.83973
Variant III Variant I	-0.475	0.22767	-2.08636	0.05443	0.05	0	-0.96027	0.01027
	-0.1	0.22767	-0.43923	0.66675	0.05	0	-0.58527	0.38527

*Tretman / Fisher Test / Analysis of the differences between the categories with a confidence interval of 95%

The total fruit weight per tomato plant was greatly influenced by the number of applications of fermented horsetail extract. Thus, the third and fourth variants had the highest fruit weight, i.e. Variant III - four weeks after the first treatment 1346.8 g/plant and Variant IV - six weeks after the first treatment 1275.4 g/plant, which was almost twice as much fruit in compared to the control (untreated) variety, where the average fruit weight was 664.9 g/plant. This confirms the fact that horsetail is used against cryptogamic diseases in soil and plants (mildew, blast, rust, moniliasis, leaf spotting and blistering, etc.). For applying this treatment, the decoction is diluted with water (one part of decoction for 5 parts of water). Treatments during the plant growing period are applied before the disease appears (1 treatment) and several times in spring and summer. Decoction diluted (50 g/l water) is also recommended for treating seeds (especially leguminous seeds) against plants (samplings) falling (melting). Fermented macerate of horsetail, combined with potassium soap (0.3%), is used against pests and for invigorating the plants [15].

Conclusions

Horsetail fermented extract may be used as fungicide for preventing tomato late blight, especially if we know that it is allowed by the EU for use in organic production. Based on this and some previous research, horsetail can be used as a substitute for copper-based products and a copper substitute. In the coming period, expand the research to a greater number of years of testing, to a greater number of treatments, as well as to the introduction of different types of herbal preparations (tea, broth, and macerate) based on horsetail.

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