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The effect of application of organic biostimulant "Zlatno inje" on the occurrence of lemon balm septoria leaf spot (*Septoria melissae* Desmazieres)

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ABSTRACT

This study examines the impact of the application of organic biostimulant "Zlatno inje" on the occurrence of lemon balm septoria leaf spot (*Septoria melissae* Desmazieres). This paper aims to find a possible solution for one of the most important diseases of cultivated lemon balm in our region from the economic point of view. This disease is caused by the fungus *S. melissae*, which can occur in the production of seedlings, but also on the plants in old plantations. The study was conducted in the experimental field of the Institute for Medicinal Plants Research "Dr Josif Pančić", which is located in Pančevo, Serbia (44°52'20"N; 20°42'06"E; 74 MASL). The experiments were conducted during the growing season in 2018 on a three-year-old plantation by a randomized block design with four repetitions. The soil type at this location is humogley (black soil). The plot size was 8.4 m^2 and the effect of the organic biostimulant "Zlatno inje" was estimated according to a scale of 1 to 5 of the presence of the disease on the lemon balm leaf. The "Citron" variety of the lemon balm was used as the herbal material. The "Zlatno inje" biostimulant was used at rate of of 3 l ha^{-1}, and it was applied in three variants and fourth variant was the control variant without the application the bio-stimulator.

Following the evaluation of the plant infection by *Septoria*, the best results were shown by the third variant, with the least presence of *Septoria* (the average of 2.1 index points), followed by the second variant on the second place, the first variant on the third place and, on the forth place, the fourth (control) variant (without the use of the bio-stimulant), where there was the highest presence of the examined disease (the average of 4.2 index points). The yield of lemon balm dried leaves was determined on the basis of the removal of aboveground mass of each elementary plot.

Key words: lemon balm, leaf spot, septoris, *Septoria melissae*, biostimulant

INTRODUCTION

Lemon balm (*Mellisa officinalis* L.) is one of few plant species with great variety of uses, having its applications as a medicinal, aromatic, decorative, spice, honey and industrial plant (Filipović & Ugrenović, 2019). It is a perennial plant belonging to the Lamiaceae family. In the last few years has been sought as a raw material for the production of essential oils, as well as fresh (for making iced teas and cocktails) and dried (for the production of monocomponent teas and tea mixtures). For medicinal purposes, it is used for its dried leaves (*Melissae folium*), for the aboveground part of the plant (*Melissae herba*) and for its essential oil (*Melissae aetheroleum*). Further, as it is a perennial plant, it is necessary to follow the guidelines regarding good producers of lemon balm production practices and methods used to improve the existing cultivation technology (Filipović & Ugrenović, 2019).

According to the list of economically harmful organisms in Serbia (the Official Gazette of the Republic of Serbia, Number 25/2008), *Septoria melissae* Desmazieres, causal agent of septoria leaf spot in lemon balm, is marked as an economically important pathogen. This pathogen is mostly located on the leaves of lemon balm (Machowicz-Stefaniak et al., 2002), and it is widely spread, especially on cultivated lemon balm (Nagy & Horváth, 2010; Jadczak & Pizoń, 2017; Wielgusz & SeidlerŁożykowska, 2017; Filipović & Ugrenović, 2019). Nagy (2002) finds that in Hungary the first symptoms of this pathogen appear on older leaves in May, but some varieties of lemon balm have proved more resistant compared to others (Kovács et al., 2019). Nevertheless, each year the presence of this pathogen affects the achieved yield and quality of lemon balm leaf.

In order to implement integrated measures, various types of biopreparations are increasingly used in plant production as nutrition and plant protection products. One of those is biostimulant "Zlatno inje", an organic soil enhancer made from natural substances of organic origin. The use of "Zlatno inje" significantly increases the yields, plant resistance to pathogens and drought (Jovović, 2011). In previous studies, the use of "Zlatno inje" had a positive effect on various parameters in a number of plant species (Nikolić et al., 2010; Jovović, 2011; Dragičević et al., 2015).

In this regard, and in order to ensure the expected quantities of lemon balm leaf with the required quality, the potential effect of the organic biostimulant "Zlatno inje" was examined in the protection of lemon balm plantations from septoria leaf spot (*S. melissae*), and presented in this study.

MATERIAL AND METHODS

The experimental research of lemon balm was carried out during the vegetation season in 2018. The location where the experiment was conducted was the seeds production unit of the Insitute for Medicinal Plant Research "Dr Josif Pančić", which is situated in Pančevo (44°52'20"N; 20°42'06"E; 74 meters above sea level). The soil type which the experiment was conducted on humogley (black soil). The soil has the following agrochemical properties: pH in KCl = 5.4, humus content = 2.3%, P_2O_5 content = 3.6 mg per 100 g of soil and K_2O = 36.2 mg per 100 g of soil.

The plant material used for the purposes of this research was lemon balm (*M. officinalis* L.), a perennial herbaceous species belonging to the Lamiaceae family, particularly, the "Citron" variety of lemon balm. The used fresh and dried leaf (*M. folium*) is official plant material by the European Pharmacopoeia (Ph Eur, 2011).

The field micro-experiments were set by a random block system with four repetitions. The size of the basic plot was 8.4 m^2 (2.8 m × 3 m). The elementary plot served as a trial plot at the same time. The experiment was based on a three-year-old plantation.

During the vegetation period of lemon balm, one feed took place immediately before the first hoeing with nitrogen mineral fertilizers (35 kg ha^{-1} N).

The experimental method was based on the monitoring of the application of registered biostimulant "Zlatno inje" (SZR „Eko-Natura-Hipokratika-Sunce", Kragujevac, Serbia) in the amount of 3 l/ha. During the experiment, three variants were applied with the use of that bio-stimulant: The first variant was the treatment application at the onset of the occurrence of the disease. The second variant was applied at the onset and two weeks after the onset of the disease. The third variant took place at the onset of the disease, then two weeks after the onset of the disease, and finally, four weeks after the onset of the disease. The fourth variant was the control one, that is, the variant without the application the bio-stimulator. During the last treatment, the evaluation was performed of the plant infection by *Septoria* on a scale of 1 to 5 index points, number 1 representing the lowest susceptibility, and number 5 pointing to the most sensitive stage of the disease. On the disease scale from 1 to 5, number 1 = no infection, number 2 = 1-25% of leaf area blighted, number 3 = 26-50%, number 4 = 51-75%, number 5 = 76-100% of leaf area blighted (Rahayu, 2014).

The yield was determined on the basis of the removal of the aboveground mass of 8.4 m^2 (2.8 m × 3 m) at each treatment. After drying and reducing the moisture to

10%, the dry leaf mass was measured and calculated. The effects of biostimulant were estimated based on the obtained dry biomass of the above ground plants part (yield), expressed per unit of soil surface (kg ha^{-1}).

The meteorological data for the vegetation period were obtained from the meteorological station of the "Tamiš" Institute, Pančevo (Table 1).

The experimentally obtained data were subjected to the two-way analysis of variance (ANOVA) using a statistical system (STATISTICA, 2010) and significance ratings derived from Duncan's test for a significance threshold of 5%.

RESULTS AND DISCUSSION

The evaluation of the infection by lemon balm septoria leaf spot and the effect of organic biostimulant „Zlatno inje" on the appearance of the causal agent of *S. melissae* are shown in Table 2.

Table 1. Meteorological data for vegetation period in 2018.

Year	Monts	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	**Σ**	**x̄**
2018	P	42.6	40.0	63.4	38.6	47.4	168	41.6	39.4	34.2	2.6	0.0	20.8	**538.6**	–
2018	T	3.2	1.7	5.4	17.1	20.6	21.7	22.5	24.6	18.9	14.9	7.7	1.4	–	**13.3**
2004-2017	P	48.2	43.3	42.7	52.1	72.8	79.3	61.5	51.2	68.0	50.9	49.6	53.1	**672.7**	–
2004-2017	T	0.8	3.0	7.5	13.1	18.4	22.0	23.8	23.5	18.1	12.5	7.1	2.4	–	**12.7**

P - Precipitation (mm); T - Temperature (°C)

Table 2a-c. Analysis of variance of the evaluation of the leaf spot infection in lemon balm

a)

Source	DF	SS	MS	F	Pr > F
Variant	3	9.5919	3.1973	20.6000	< 0.0001
Error	12	1.8625	0.1552		
Corrected Total	15	11.4544			
Computed against variant Y=Mean(Y)					

b)

Contrast	D	SD	CV	Pr > Diff	α (Modified)	Sign
IV CTRL vs III VAR	2.0750	7.4486	2.3423	< 0.0001	0.1426	Yes
IV CTRL vs II VAR	1.6250	5.8333	2.2806	0.0002	0.0975	Yes
IV CTRL vs I VAR	1.3750	4.9358	2.1788	0.0004	0.0500	Yes
I CTRL vs III VAR	0.7000	2.5128	2.2806	0.0656	0.0975	Yes
I VAR vs II VAR	0.2500	0.8974	2.1788	0.3871	0.0500	No
II VAR vs III VAR	0.4500	1.6154	2.1788	0.1322	0.0500	No

CTRL - control; VAR - variant; D - difference; SD - standardized difference; CV - Critical value

c)

Category	LS means	Groups		
IV CTRL	4.2000	A		
I VAR	2.8250		B	
II VAR	2.5750		B	C
III VAR	2.1250			C

Treatment / Duncan / Analysis of the differences between the categories with a confidence interval of 95

The best results, i.e. the lowest intensity of the infection by leaf spots, were achieved in the third variant, where lemon balm was treated three times with the selected organic bio-stimulant (at the onset of the disease, then two weeks after the onset of the disease, and finally, four weeks after the onset of the disease). The rated plants averaged 2.1 index points, which is the lowest susceptibility to the occurrence of this economically significant disease of lemon balm. The highest occurrence of the disease was observed in the fourth (control) variant, (the variant without biostimulant application), where the presence of leaf spot had a value of 4.2 index points, making lemon balm the most susceptible to the occurrence of leaf spot, i.e. the *S. melissae* fungus. In that regard, the analysis of variance revealed significant differences at all levels of significance as a result of the effect of the tested treatments on the occurrence of that economically significant fungal disease (*F*-test = 20-600**, p≤0.01 for the first-order interaction).

In the research study by Kowalska et al. (2014), it was found that the effect of *Trichoderma asperellum* had a direct antagonistic effect on the presence of the *S. melissae* fungus. In the research study by Machowicz-Stefaniak et al. (2002), the dominant fungus, mostly populating lemon balm leaves in comparison with other plant parts, was the polyphage fungus *Alternaria alternata* (Fr.) Keissler, which, in certain conditions, can greatly affect the condition of the plantation itself, but also the productive and qualitative properties of the cultivated lemon balm. The presence of the *S. melissae* fungus was also noticeable. In addition to a certain tolerance when selecting suitable varieties for this purpose, the use of certain preparations can reduce to a certain extent the negative effect by this fungus on the required market parameters (Filipović & Ugrenović, 2019). In Poland, when applying the use of seven fungicide species, the presence of the disease *S. melissae* was in the range of 45.22 to 78.45% (Mikolajewicz & Filoda, 1998).

Crucially important in this experiment were: the degree of *Septoria* leaf infestation, the use of the "Zlatno inje" biostimulant and the weather during the growing season. The lowest yield of fresh as well as dried leaves was achieved in the control variant (variant with no treatment). In that "untreated" variant, 7424 kg ha^{-1} of fresh leaf and 1738 kg ha^{-1} of dry leaf were recorded, which is significantly less than the third variant tested, where 10518 kg ha^{-1} of fresh leaf and 2129 kg ha^{-1} of dry leaf were obtained (Tables 3 and 4).

Table 3a-c. Analysis of variance of the effect of the "Zlatno inje" biostimulant on the yield of fresh lemon balm leaf (kg ha^{-1})

a)

Source	DF	SS	MS	F	Pr > F
Variant	3	22872821.1875	7624273.7292	12.8536	0.0005
Error	12	7117958.7500	593163.2292		
Corrected Total	15	29990779.9375			
Computed against variant Y=Mean(Y)					

b)

Contrast	D	SD	CV	Pr > Diff	α (Modified)	Sign
III VAR vs IV CTRL	3093.7500	5.6808	2.3423	0.0005	0.1426	Yes
III VAR vs I VAR	2642.7500	4.8527	2.2806	0.0011	0.0975	Yes
III VAR vs II VAR	1492.7500	2.7410	2.1788	0.0179	0.0500	Yes
II VAR vs IV CTRL	1601.0000	2.9398	2.2806	0.0309	0.0975	Yes
II VAR vs I VAR	1150.0000	2.1117	2.1788	0.0564	0.0500	No
I VAR vs IV CTRL	451.0000	0.8281	2.1788	0.4238	0.0500	No

CTRL - control; VAR - variant; D - difference; SD - standardized difference; CV - Critical value

c)

Category	LS means	Groups		
III VAR	10517.7500	A		
II VAR	9025.0000		B	
I VAR	7875.0000		B	C
IV CTRL	7424.0000			C

Treatment / Duncan / Analysis of the differences between the categories with a confidence interval of 95

Table 4a-c. Analysis of variance of the effect of the the "Zlatno inje" biostimulant on the yield of dry lemon balm leaf (kg ha^{-1})

a)

Source	DF	SS	MS	F	Pr > F
Variant	3	331860.5000	110620.1667	28.9181	< 0.0001
Error	12	45903.5000	3825.2917		
Corrected Total	15	377764.0000			
Computed against variant Y=Mean(Y)					

b)

Contrast	D	SD	CV	Pr > Diff	α (Modified)	Sign
III VAR vs IV CTRL	391.2500	8.9462	2.3423	< 0.0001	0.1426	Yes
III VAR vs I VAR	284.0000	6.4938	2.2806	< 0.0001	0.0975	Yes
III VAR vs II VAR	184.7500	4.2244	2.1788	0.0012	0.0500	Yes
II VAR vs IV CTRL	206.5000	4.7217	2.2806	0.0013	0.0975	Yes
II VAR vs I VAR	99.2500	2.2694	2.1788	0.0425	0.0500	Yes
I VAR vs IV CTRL	107.2500	2.4523	2.1788	0.0305	0.0500	Yes
CTRL - control; VAR - variant; D - difference; SD - standardized difference; CV - Critical value						

c)

Category	LS means	Groups			
III VAR	2129.0000	A			
II VAR	1944.2500		B		
I VAR	1845.0000			C	
IV CTRL	1737.7500				D
Treatment / Duncan / Analysis of the differences between the categories with a confidence interval of 95					

However, in the case of a more sensitive variety, cultivation on a less suitable soil type and in unfavorable weather conditions, the occurrence and intensity of the presence of lemon balm leaf spot will be higher and the productivity and quality of the raw material obtained will be lower (Németh-Zámboriné et al., 2015). In the northern parts of India, the highest biological dry leaf yield of 6,788 kg/ha was obtained using 90 kg N/ha (Abbaszadeh et al., 2009). The positive effect of the application of certain amino acids on the yield of lemon balm may be due to the lack of carbohydrates, amino acids "taking over" their role as a source of energy (Mehrafarin et al., 2015).

CONCLUSIONS

Based on the results of the presented research, the positive effect of the application of the organic bio-stimulant "Zlatno inje" on the occurrence of the causal agent of the leaf spot with lemon balm, *S. melissae*, on the yield of fresh and dried lemon balm leaf, we can come to the following conclusion:

The most effective variant in suppressing this disease, economically significant for lemon balm, was the third variant, when lemon balm was treated three times with the selected organic bio-stimulant (at the onset of the disease, two weeks after the disease had showed up, and then, four weeks following the onset of the disease). The most adverse results, as expected, were recorded in the control variant, in which the intensity of the infection was the highest.

The highest yield of fresh as well as dried leaves of lemon balm was also recorded in the same variant. Compared to the first variant (one treatment at the onset of the disease) and the fourth (control) one, the second variant (one treatment at the onset of the disease and two weeks after the disease had showed up) and third one had significantly higher yields. This makes the use of an organic bio-stimulant additionally justified.

Further research regarding the effect of the application on the emergence of lemon balm leaf spots (*S. melissae*) should be directed towards the research of available plant protection products and nutrients, especially those named on the List of Plant Protection Products and the List of Plant Nutrition Products and soil enhancers that can be used in organic production, updated by the Working Group appointed by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia.

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