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DISRUPTION OF ATTRACTANT PROPERTIES OF POTATO FOLIAGE ON *LEPTINOTARSA DECEMLINEATA* SAY BY THE USE OF *SALVIA OFFICINALIS* L. ESSENTIAL OIL

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SUMMARY

Colorado potato beetle has a very high reproductive potential in Serbia due to favorable climatic conditions and nutrition. Considering that it is an introduced species, the activity of limiting biotic factors seems negligible. With the time, it developed resistance on commercial insecticides, and consequently they cease to be used for this purpose. In recent years, not only in the EU, a strong public pressure has been posed on production of safe food, i.e. food with no pesticide residues which are harmful and dangerous to human health. For these reasons, today we examine several alternative routes, such as: the creation of resistant potato varieties (transgenic potatoes), joint cultivation of potatoes with plants with efficacy to repel or confuse the potato beetle. A great number of herbal extracts and essential oil has been studied so far in order to find natural solution that will replace conventional insecticides. In this study, the possibility to disrupt the attractant properties of potato leaf on Colorado potato beetles, by application of 95% ethanol solutions of sage essential oil and its fractions (F1-F5) (0.5% concentration) was examined. Chemical composition of sage essential oil and its five fractions used in experiment was presented. Tests were conducted at the Adult (female) *Leptinotarsa decemlineata* Say in the olfactometer. The experiments were set up in five replicates, in micro-climatic chamber, with following constant conditions: temperature 27°C ± 1°C, relative humidity 65% ± 5% and the illumination was 9400π cd. Results were processed by the use of Analysis of variance and Duncan's test. The most pronounced disturbance was recorded with application of sage essential oil and the least one was achieved with application of the fraction one (F1). The obtained results indicate possibility of using secondary metabolites of *Salvia officinalis* L. in protection of potato foliage of its major pest (*L. decemlineata*).

Keywords: *Leptonotarsa decemlineata* Say, sage essential oil, fractions, disturbance, olfactometer.

INTRODUCTION

Colorado potato beetle (*Leptinotarsa decemlineata* Say) has a very high reproductive potential in Serbia due to favourable climatic conditions and nutrition. Considering that it is an introduced species, impact of limiting biotic factors seems to be negligible. With the time, the pest has developed resistance on commercial insecticides, so they cease to be applied for this purpose. In addition, they proved to be highly toxic to non-target organisms, including humans and the environment [1, 2, 3], and since they are not easily biodegradable, they also leave dangerous residues in food products.

In recent years, not only in the EU, a strong public pressure has been posed on production of safe food, i.e., food without pesticide residues harmful to human health. For these reasons,

several alternative approaches have been examined, such as: the creation of resistant potato varieties (transgenic potatoes), joint cultivation of potatoes with plants with efficacy to repel or confuse the potato beetle.
A great number of herbal extracts and essential oil has been studied so far in order to find out natural solution that will replace conventional insecticides. In this study, the possibility to disrupt the attractant properties of potato leaf on *Leptinotarsa decemlineata* Say female adult, by application of *Salvia officinalis* L. essential oil and its fractions (F1-F5) was examined in olfactometer.

MATERIALS AND METHODS

Experimental design

Essential oil of *Salvia officinalis* L. was isolated by the Clevenger steam distillation apparatus. The oil and its five fractions (F1–F5) were subjected to detailed qualitative and quantitative analysis using the GC and GC/MS method. Chemical composition of sage (*Salvia officinalis* L.) oil and its five fractions (F1-F5) are presented in Kostic et al., 2007 [4].Prior to be applied, the oil and the fractions were dissolved in 96% ethanol. The repellent efficacy of 0,5% solutions of the oil and it's F1–F5 fractions on *L. decemlineata* was examined.

Insect Colorado potato beetle (Leptinotarsa decemlineata Say)

The insects originated from Dobanovci (Serbia). The insect population proved to be resistant to carbamates and ogranophosphorous compounds [4] was used.Insects were collected from the potato not treated with pesticides. The insect population was grown in the laboratory conditions. The experiments were conducted in five replications and for each replication new adults were used. Only adult female individuals (3 days old) of Colorado potato beetle were used, since they are responsible for discovering the host plants, enabling the survival of the species. In the experiment, opportunity to choose between the discs of potato leaf treated with 0,5 % solution (different variants) and untreated leaf disk (control treatment), was provided to female adults, in order to examine possibility to prevent the pest to discover potato leaf. Since the adults in experiment used to easily discover untreated leaf disks, time and speed they needed to complete the task were recorded.

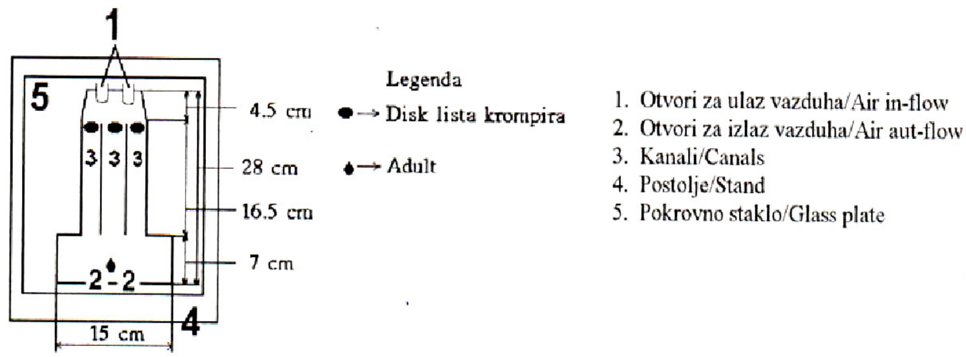
Potatoes

Potato cultivar “Dessire” was grown in greenhouse conditions, without application of pesticides. Fresh potato leaf disks, 2 cm diameter, were first immersed in 0,5 % solution (weather of sage essential oil or one of its five fraction), then dried for 20 minutes at 27°C, and finally placed in the olfactometer (in the left tunnel of the olfactometer).

Olfactometer

To test the repellent efficacy of the 0,5% solutions of sage essential oil and its fractions, olfactometer was used. The olfactometer is made of 3-5 mm thick glass, and consists of two entrances for the air intake and two exits of the exhausted air. The air passes through three tunnels (17cm long and 3,5cm wide). The air comes to the part where the olfactometer is expanded and it has two openings (related to the part with tunnels), in order to prevent the air turbulence (dimensions of this part of olfactometer are 15x7cm). The overall dimensions of usable space in the olfactometer are 28x15x5cm (length x width x height).

Figure 1. Schematic display of olfactometer used in experiment.



In order to function, the olfactometer uses the air pump and rubber-coated tubes (9 mm in diameter) for the inlet air to the manifold, the air flow regulator, the rotameter and the glass air hub with activated charcoal (which served to neutralize the odoriferous substances in the air). The air velocity through the rotameter was ca. 2000 l/ h (i.e. 0,55 l/s), while in the olfactometer itself the air speed was ca. 75 ml/s.

Distance between the potato leaf discs and the adults was 21 cm. In the left tunnel, treated potato leaf disk (various solutions) was placed while the untreated leaf disk was placed in the right tunnel. The adults' preferences towards treated/untreated potato leaf disks, as well as their timing to reach the leaf disks, was monitored.

Air Chamber

The experiments were placed in air-chamber in order to enable optimal conditions for the biological cycle of the Colorado potato beetle. Temperature and relative humidity was adjusted via control panel ("Danfoss, EKH 20"). All experiments were carried out in micro-climatic chamber under following stable condition: T = 27±1°C, Relative humidity (RH) = 65±5% and illumination 9400 candela π.

Statistical..analysis

Results were analyzed using Analysis of Variance and statistical significances determined by the Duncan test.

RESULTS AND DISCUSSION

Chemical composition of sage essential oil showed presence of 14 different compounds. The main ones were α–thujone (31.87%) and camphor (24.65%). Each of the sage oil fraction (F1–F5) showed less number of compounds with their different presence (%) in the fraction, in comparison to the entire sage essential oil. The F-1 fraction had 8 compounds, with dominant one being α–thujone (25.37%). The F-2 fraction had 7 compounds and the dominant one was also α–thujone (48.99%). Both fractions, F-3 and F-4 had 6 compounds, with dominant one being camphor (46.99% and 44.42%, respectively), while the fraction F5 had 7 compounds with two dominant compounds with similar presence; γ–selinen (19.57%) and α–humulene (18.27%). The only component that is present in the entire sage oil and in all five fractions of the sage oil was camphor and with the highest percentage detected in the F-3 fraction (46,99%). Chemical composition of the entire sage oil and the five sage oil fractions (F1-F5) used in experiment are presented in Table 1.

Analysis of variance revealed that very significant differences ($p \leq 1\%$) were recorded between the time length felame adults needed to reach untreated and treated leaf discs, depending on the applied treatment (Table 1).

Table 1. Analysis of variance for the time (in seconds) Colorado potato beetle female adults need to reach untreated potato leaf disks, depending on the examined variants.

Source of variation	Degrees of freedom	Mean square	F value
Repetitions	4	119.95	2.16
Variants	5	8394.85	151.34**
Error	20	55.47	
Total	29		

** ≤ 1%

The fastest average arrival time to the untreated potato leaf disk (35,6 s) was recorded for adults that had to choose between the untreated leaf disk and the leaf disk treated with sage essential oil. The average times of adults to reach untreated disks in experiment with fractions F3, F4 and F5 were similar (65.0–72.6 s), while in the experiment with fractions F2 and F1, adults needed much more time (85.8 and 157.6 s, respectively). The results are presented in Table 2.

Table 2. Time (in seconds) that Colorado potato beetle female adults needed to arrive to the untreated potato leaf disc, depending on the examined variants.

Treatments	Repetitions					Average values
	I	II	III	IV	V	
Sage ess. oil (*S. officinalis*)	26	35	38	37	42	35.6**a**
Fraction 1	158	155	158	164	153	157.6**d**
Fraction 2	88	80	84	88	89	85.8**c**
Fraction 3	52	74	70	84	83	72.6**b**
Fraction 4	67	68	60	72	84	70.2**b**
Fraction 5	68	50	75	67	65	65.0**b**

$LSD_{0.05}$ = 9.8 seconds

The process of selection of the host plant by insects is composed of three quite distinct phases: 1. Insects has to be attracted by potential host plants; 2. Insects has to reach the host plant; 3. Stimulation (prevention) of the insect to feeding on the host plant [5]. This confirms that there is a subtle interaction between the host plants and insects, mainly achieved by their sensory organs, especially those used to establish their contact with given environment and feel smell and taste (*sesilum basiconicum*).

Plant species are abundantly present on Earth and are preferred by many pathogenic organisms. It is assumed that there are 6 million species of organisms, 50% of them being harmful to plants [6]. In order to effectively fight against the invasion of microbial pathogens and insect herbivores, plants have developed sophisticated defensive strategy to "see" the attack of insects and other pathogens, and thus translate this "experience" in appropriate defensive response [7, 8]. These defensive responses helped plants to develop their defense by networking of interconnecting signals of different plant components produced by the plants in order to protect themselves from harmful pathogens [9, 10]. On the attacked places, the plant accumulate metabolites as a response to pathogen infection or damage caused by phytophagous insects, by activating different sets of defense associated with genetic inheritance [11]. For these reasons, scientists intensively search for natural substances that have possibility to deter insects not to come and feed on host plants (repellents and antifeedants) nor to place their embryos (anti-oviposition), which also have no toxic effects (contact or gastrointestinal) so they could be used in the plant protection [12].

It was found that the main components, responsible for the attractive effect on the Colorado potato beetle, are present in the essential oil of potato leaves. The basis of this "grass-like" smell the green potato leaf volatiles represents a chain of saturated and unsaturated aldehydes and alcohols produced by oxidative degradation of plant lipids. The relative proportion of these final products varies among different plant species within the same genus, and also seasonally, within a single species, and due to plant aging or injuring, all of these affecting the degree of attraction of Colorado potato beetle by the plant [13, 14, 15, 16]. Visser et al. [17] determined following volatile components present in potato leaf that attract Colorado Potato beetle, *trans*-2-hexene-1-ol, 1-heksenol, *cis*-3-hexene-1-ol, *trans*-2-heksenol and linalool, present in potato leaves in following percentage proportion: 100: 17 : 17 : 7: 4, respectively.

In our experiment, all tested variants successfully deterred the arrival of female adults of Colorado potato beetle on the treated potato leaf disks. The difference between different variants applied was recorded in the time they needed for arrival to the untreated disk. The fastest arrivals to untreated leaf disk have had those that had to choose between the leaf disk treated with sage essential oil and untreated leaf disks (the average arrival time was 35.6 sec). The most of the measured time adults have spent on making correct choice between the untreated leaf disks and disk treated with fraction F1 (157.6 sec). Regarding the remaining fractions F2, F3, F4 and F5, the adults spent the shortest time to reach untreated leaf disks when fraction F5 is applied (65.0 sec), while when other fractions were applied the required time ranged between 70.2 and 85.8 seconds.

Despite the attempts to mask the attractant property of potato foliage on *L. decemlienata*, by the use of various herbal extracts and/or essential oils, in order to prevent them to find their host plant, a lot of efforts has beet input in order to find out how to prevent these pests to feed on [18] or how to prevent both, feeding and ovipositioning on potato foliage [19].

CONCLUSION

In this study the possibility of preventing the female adults of Colorado potato beetle to feed on their host plant potato by attempting to mask the attractant volatile components of potato foliage with 0.5% ethanol solution of sage essential oil (*S. officinalis*) and its five fractions (F1-F5) was examined The application of sage oil caused the most significant interference, followed by the application of the fraction F5. The obtained results encourage further researches on disturbance of the attractant properties of potato foliage volatiles on Colorado potato beetle female adults or prevention of the arrival of adults on the host plant (first phase), with the use of various concentrations of sage essential oil and its fraction F5.

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