

## SCREENING TEST FOR DETECTION OF COLORADO POTATO BEETLE (*LEPTINOTARSA DECEMLINEATA* SAY) SENSITIVITY TO ABAMECTIN\*

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*SUMMARY: Screening test was carried out to assess the sensitivity to 19 filed populations of Colorado potato beetle (*Leptinotarsa decemlineata* Say.) to abamectin during 2008 (Futog, Čenej, Vilovo, Kovilj and Čurug), 2009 (Stepanovićevo, Gložane, Žabalj, Sirig, Šajkaš, Ljutovo, Šimuže, Bačko Gradište, Novo Miloševo i Kikinda) and 2012 (Zmajevno, Despotovo, Vilovo and Čurug). The test provided a quick assessment of sensitivity of overwintered adults to abamectin (commercial insecticide Abastate) applied at label rate (0.75 l/ha), two-fold (1.5 l/ha) and five-fold higher rates (3.75 l/ha), by soaking method (5 sec). Mortality was assessed after 72 h of exposition. Results were corrected for the mortality in the control and presented as efficacy (%) after the treatment. According to IRAC (method 011), with slight corrections, the sensitivity of Colorado potato beetle was classified by scale 1-5 (1 - highly sensitive population ( $E = 100\%$ ); 2 - sensitive ( $100 > E \geq 95\%$ ); 3 - slightly resistant ( $95 > E \geq 90\%$ ); 4 - resistant ( $90 > E \geq 50\%$ ); 5 - highly resistant ( $E < 50\%$ ). From five tested populations in 2008, four were highly sensitive ( $E = 100\%$ ) to abamectin at label rate and one was sensitive ( $E = 97.5\%$ ). In 2009, out of ten tested populations, three were slightly resistant ( $E = 92.6-94.6\%$ ), five were resistant ( $E = 64.6-88.7\%$ ) and two were highly resistant ( $E = 22.5-48.3\%$ ) to abamectin at label rate. Out of four tested populations in 2012, one was highly sensitive ( $E = 100\%$ ), one was sensitive ( $E = 95.8\%$ ) and two were slightly resistant ( $E = 90.6-94.2\%$ ). The survival of adults in treatments with higher rates of abamectin, that the label one, prove that there is a part of population which has endured changes in susceptibility or selection to resistance.*

**Key words:** screening test, Colorado potato beetle, sensitivity, abamectin.

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## INTRODUCTION

Pesticide use in agriculture has, aside from well known advantages, caused some negative effects in the course of time. Such are changes in sensitivity of harmful insects, or better known, their resistance to insecticides. The most frequently mentioned example for the resistance occurrence is Colorado potato beetle (CPB), which represents the most harmful species in potato production.

Considering the facts about the share of potato in human nutrition, amounts and diversity of pesticides (insecticides, fungicides, herbicides) used for protection of this crop even in storage process, and the quality of this feed stuff, the risk of pesticide residues is of great importance (Indić et al., 2006a). To ensure the expected yield in Serbia potato crops are treated with insecticides two to four times during vegetation (Zabel et al., 2000). According to several authors, CPB resistance to carbamates and organophosphates, as well as to pyrethroids (Indić, 1997; Perić et al. 1997) was registered in a number of populations in Serbia (Indić, 1997; Perić et al., 1997; Stanković et al., 2004; Indić et al, 2006; Indić et al., 2009; Marčić and Perić, 2009; Indić et al., 2012). Literature data indicate that CPB resistance was noted for 42 insecticides, belonging to different chemical groups (organophosphates, carbamates, pyrethroids), which justifies the fact of CPB ranking among 10 species that most rapidly develop resistance (Mota-Sanchez et al., 2006; Whalon et al., 2008). In practice, the problem of resistance in our region is resolved in accordance with principles of integrated pest management using different measures (manual collection, rarely bioinsecticides and growth regulators) which reduces the selection pressure of insecticides to insects. However, farmers that have large areas under potato production, mostly use chemical measures. Thus, within the strategy of delay or slowing down the resistance process it is recommended to use mixtures of insecticides with different modes of action. Although, other measures are familiar, such as predators and parasitoids, plant extracts, joined cropping (Copping and Menn, 2000; Gokce et al., 2006), the selection of potato varieties to CPB resistance, or spatial distance of areas under potato production, which directly or indirectly reduce selection pressure of insecticides, they are not common in practice.

The aim of the study was the creation of simple and rapid test for detection of sensitivity levels of CPB to insecticides and confirmation of the resistance, as well as providing simplified presentation of the results. The method is based on the assessment of sensitivity of overwintered adults to insecticides. It allows a quick formation (24-72 h) of information network related to production regions and on these bases further establishment of strategy for rational use of insecticides in CPB control. However, the insight into the part of population which survived the insecticides applied at label and higher rates, allow us to make statement that a certain part of population has endured changes in sensitivity.

## MATERIAL AND METHODS

The site selection. For monitoring of CPB sensitivity to insecticides, based on the advice of experts from Agricultural extension service, 19 sites on the territory of Serbia during 2008 (Futog, Čenej, Vilovo, Kovilj and Čurug), 2009 (Stepanovićevo, Gložane, Žabalj, Sirig, Šajkaš, Ljutovo, Šimuže, Bačko Gradište, Novo Miloševo and Kikinda) and 2012 (Zmajevo, Despotovo, Vilovo and Čurug) were selected and marked with GPS

coordinates.

Applied insecticide. Insecticide Abastate (a.i. abamectin) was applied at 0.75 l/ha – label rate; 1.5 l/ha – two-fold label rate and 3.75 l/ha – five-fold label rate.

Test insect. This bioassay included overwintered adults of CPB field populations, which were not in direct contact with insecticides prior to testing. Insects were kept in laboratory conditions, without additional feeding, at temperature of  $23 \pm 2$  °C and normal photoperiod (16/8h).

Toxicological experiment. Bioassay was based on the assumption that CPB populations have reduced sensitivity to insecticides and on evaluation of response of overwintered adults to insecticide applied at label rate (the rate determined in field experiments during the registration process and found to cause 100% mortality) and higher rates. Insecticides were applied by insects soaking for 5 sec. The experiment was set up in four replicates with 30 adults per replication (sex ratio 1:1). The sex was determined according to Tribelj and Korol (2001). Assessment of insecticide efficacy consisted of counting the number of dead (with no signs of vitality), paralyzed (uncoordinated movements and inability to move) and alive insects (normal mobility and vitality). The effect was determined 24, 48 and 72 h after insecticide application. Results were corrected for mortality in the control (Schneider Orelli, 1947) and expressed as the efficacy (E%) achieved only after 72 h. Sensitivity was evaluated on the scale 1-5 (1 - highly sensitive populations /E = 100%/; 2 - sensitive /100> E ≥ 95%/; 3 - slightly resistant /95> E ≥ 90%/; 4 - resistant /90> E ≥ 50%/; 5 - highly resistant /E <50%/), which was created as a slight modification of IRAC method No. 011 (Anonymous, 2009) that refers to pollen beetles (*Meligethes* spp.).

## RESULTS AND DISCUSSION

In our conditions abamectin is used less frequently for the control of the Colorado potato beetle, compared to insecticides from organophosphates, carbamates and synthetic pyrethroids chemical groups.

Abamectin is an analog of a metabolite produced by fungus *Streptomyces avermitilis*, which is according to IRAC - classified into the group avermectins, and exhibits insecticidal and acaricidal activities. It has contact and digestive action. Since it is agonist of  $\gamma$  agonist - aminobutyric acid, the mechanism of action is based on the interruption of neurotransmission. Toxicity is manifested primarily in cease of insect feeding and afterwards in paralysis of irreversible character (Janjic and Elezović, 2008). Stumpf and Nauen (2002) mention the possibility of different biochemical and pharmacokinetic mechanisms being responsible for the emergence of resistance to abamectin in target organisms. In resistant Colorado potato beetle races to abamectin, the enhanced enzyme activity is registered (carboxylesterase and monooxygenase), as well as the increased metabolism and excretion of this compound. In our conditions, abamectin is relatively rarely used for the control of Colorado potato beetle, compared to insecticides belonging to the group of organophosphates, carbamates and synthetic pyrethroids.

By applying the above described screening test, in our conditions, in 2007, a monitoring of CPB sensitivity towards several insecticides from different chemical groups (organophosphates, carbamates, pyrethroids, phenyl pyrazoles, abamectin) was conducted and the results are presented in several papers (Indić et al., 2009; 2012). The mentioned bioassay is based on the assumption that the populations of Colorado potato beetle have

reduced sensitivity to the insecticide. This is based on measuring the response of overwintered adults to insecticides applied at label and higher rates, but can also be used to estimate the sensitivity of the population towards newly introduced substances. The analysis of the efficacy of insecticide applied at label rate in this test, tended to provide the conditions close to the once in the field, because it is expected to have high efficiency. Otherwise it is suspected that a change in sensitivity has occurred. The application of higher rates (two- and five-fold) of the insecticide at was used to confirm that the individuals from the same population can survive even higher quantities of insecticide i.e. the evidence that the change in sensitivity has already occurred. Mentioned indicates which part of population is sensitive to label rate of insecticide, or which tolerates it, or exhibits reduced sensitivity, or that individuals have developed an appropriate mechanism of resistance. Knowing that there is no correlation between the increase in the amount of insecticides and increase mortality in the population, namely that there is a high percentage of survival of insects regardless on the rate of insecticide, and that the population has become resistant, requires: unconditional exclusion of such insecticide from the production, compliance of application strategies, regular recording or monitoring and mapping of such populations. Based on the efficacy of insecticides obtained in screening test (after 72 h exposure of insects) and in some degree modified scale for the classification of susceptibility to insecticides, a classification of CPB susceptibility to abamectin was carried out. This test can be performed in the modest experimental conditions, without special requirements for specific equipment, handling procedures are very simple and the results are clear and very applicative because they do not require complicated statistical analysis, except for precise classification of the scale 1-5. The overwintered adults were chosen as test insects in evaluation of sensitivity to insecticides for various reasons. Firstly, it is known that they were not in direct contact with insecticides, also, they are easily maintained for a long period prior to inclusion in the test, no need to be fed during the tests, and the effects of insecticides (mortality, paralysis, vitality) are easily observable and measurable.

Based on tests performed in 2008 the adults of CPB from the site Futog, Vilvo, Koviļj and Ćurug were highly sensitive to abamectin, while from Ćenej were sensitive since 2.5 % of adults survived the treatment with abamectin applied at label rate (Table 1).

The results of the sensitivity of CPB in 2009 are presented in Table 2. Three CPB populations (GloŹane, Sirig and Kikinda) were slightly resistant to abamectin applied at label rate, i.e. in mentioned populations 5.4 to 7.4 % of adults survived the treatment, and the increase of the application rate caused efficiency of 100%. Five CPB populations (Stepanovićevo, Źabalj, ŹajkaŹ, ŹimuŹe and Novo MiloŹevo) were resistant ( $90 > E \geq 50$  %), and survival rate ranged from 11.3 to 35.4 %. These populations exhibited different sensitivity to the increase of insecticide rate. Population from Stepanovićevo, even at five-fold higher rates can be classified as slightly resistant, meaning that the population has extremely heterogeneous susceptibility to abamectin. In other populations the increase of the application rate caused the increase in sensitivity. Populations from Ljutovo and B. GradiŹte were highly resistant to abamectin. The heterogeneity in sensitivity of CPB populations was noted. In the population from B. GradiŹte, after 72 hours, 77.5 % of adults survived abamectin applied at label rate, and from Ljutovo population 51.7 %. In these two populations, abamectin did not express 100 % efficacy even after the application of five-fold higher rates.

In 2012 the tests were repeated at sites Vilovo and Ćurug. Compared to the results from the 2008 the population from Vilovo, in 2012 had survival rate 9.4 %, thus can

be classified as slightly resistant, while the increase of the application rate caused the efficiency of 100 %. The level of sensitivity of the population from Čurug remained at the same level as in 2008 i.e. highly sensitive. The population from Despotovo was sensitive, with the survival rate 4.4% and the one from Zmajevu was slightly resistant, since 5.8 % of adults survived the treatment, while at higher rates than the label one, it exhibited high sensitivity (Table 3).

Similar results were obtained by Marčić and Perić (2009), when testing the efficacy of abamectin under field conditions (2004 and 2005) for the suppression of the first generation of CPB larvae. In both years, abamectin expressed high efficacy (94.8-100 %) when applied in combination with adjuvant, although the sensitivity differs depending on developmental stage and age of larvae (Indić, 1994). Test results of Mohamed (2010) in laboratory conditions show that abamectin caused high mortality (71-88.9 %) of CPB larvae ( $L_1$  -  $L_3$ ) after three days, and 100 % of mortality after seven days. The mortality of  $L_4$  larvae was 48.9 % after three days, and 100 % after seven days. The same author assessed the adulticidal effect of abamectin when applied at label rate, 50 % and 25 % lower quantities. At all applied rates abamectin achieved high mortality of adults (51.1-71.1 %) after three and after seven days (75.6-95.6 %), depending on the applied rate of insecticide.

Table 1. Sensitivity (scale 1-5) of overwintered CPB adults to **abamectin in 2008**

Sites	kg; l/ha	efficacy (%) of abamectin				
		1	2	3	4	5
Futog	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Čenej	0.75	-	97.5 (2.5)	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Vilovo	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Kovilj	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Čurug	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-

1 - highly sensitive population ( $E = 100\%$ ); 2 - sensitive ( $100 > E \geq 95\%$ );

3 - slightly resistant ( $95 > E \geq 90\%$ ); 4 - resistant ( $90 > E \geq 50\%$ ); 5 - highly resistant ( $E < 50\%$ )

Table 2. Sensitivity (scale 1-5) of overwintered CPB adults to abamectin in 2009

Sites	kg; l/ha	efficacy (%) of abamectin				
		1	2	3	4	5

Stepanovićevo	0.75	-	-	-	73.9 (26.1)	-
	1.5	-	-	-	-	37.6 (62.4)
	3.75	-	-	92.9 (7.1)	-	-
Gložane	0.75	-	-	94.6 (5.4)	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Žabalj	0.75	-	-	-	69.1 (30.9)	-
	1.5	-	-	90.8 (9.2)	-	-
	3.75	100	-	-	-	-
Sirig	0.75	-	-	92.6 (7.4)	-	-
	1.5	-	98.9 (1.1)	-	-	-
	3.75	100	-	-	-	-
Šajkaš	0.75	-	-	-	88.7 (11.3)	-
	1.5	-	-	95.6 (4.4)	-	-
	3.75	100	-	-	-	-
Ljutovo	0.75	-	-	-	-	48.3 (51.7)
	1.5	-	-	-	83.3 (16.7)	-
	3.75	-	99.2 (0.8)	-	-	-
Šimuže	0.75	-	-	-	87.2 (12.8)	-
	1.5	-	97.2 (2.8)	-	-	-
	3.75	100	-	-	-	-
Bačko Gradište	0.75	-	-	-	-	22.5 (77.5)
	1.5	-	-	-	-	25.1 (74.9)
	3.75	-	95.6 (4.4)	-	-	-
Novo Miloševo	0.75	-	-	-	64.6 (35.4)	-
	1.5	-	-	-	84.5 (15.5)	-
	3.75	-	99.1 (0.9)	-	-	-
Kikinda	0.75	-	-	93.6 (6.4)	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-

1 - highly sensitive population (E= 100%); 2 - sensitive(100 > E ≥95%);

3 - slightly resistant (95>E ≥90%); 4 - resistant (90>E ≥50%); 5 - highly resistant (E < 50%)

Table 3. Sensitivity (scale 1-5) of overwintered CPB adults to abamectin in 2012

Sites	kg; l/ha	efficacy (%) of abamectin				
		1	2	3	4	5
Zmajevno	0.75	-	-	-	-	-
	1.5	100	-	94.2 (5.8)	-	-
	3.75	100	-	-	-	-
Despotovo	0.75	-	95.6 (4.4)	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Vilovo	0.75	-	-	90.6 (9.4)	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-
Čurug	0.75	100	-	-	-	-
	1.5	100	-	-	-	-
	3.75	100	-	-	-	-

1 - highly sensitive population ( $E = 100\%$ ); 2 - sensitive ( $100 > E \geq 95\%$ );

3 - slightly resistant ( $95 > E \geq 90\%$ ); 4 - resistant ( $90 > E \geq 50\%$ ); 5 - highly resistant ( $E < 50\%$ )

## CONCLUSION

Based on the results of the screening test of sensitivity of 19 populations of CPB, in Vojvodina, to abamectin in 2008, 2009 and 2012 the following conclusions can be drawn:

- In 2008, all CPB populations (Futog, Vilovo, Kovilj Čurug ) were highly sensitive to abamectin applied at label rate, apart from Čenej population which was sensitive because the survival rate was 2.5 %;
- In 2009, three CPB populations (Gložan, Sirig, Kikinda) were slightly resistant, five (Stepanovićevo, Žabalj, Šajkaš, Šimuže, Novo Miloševo) were resistant and two (Ljutovo, B. Gradište) were highly resistant to abamectin. In all populations the manifested survival rate was ranging from 11.3 to 77.5 %;
- In 2012, the population from Čurug was highly sensitive, from Despotovo sensitive and from Zmajevno and Vilovo) were slightly resistant to abamectin, since 4.4-9.4 % of CPB adults survived abamectin applied at label rate;
- The application of this screening test provides quick and reliable measuring of which part of CPB population has endured the changes in sensitivity to tested insecticides and provide data of great importance for the extensive agricultural practices as well as for sophisticated (at the molecular level) research.

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**PRIMENA SCREENING TESTA PRI DETEKCIJI OSETLJIVOSTI  
KROMPIROVE ZLATICE (*LEPTINOTARSA DECEMLINEATA* SAY) NA  
ABAMEKTIN**

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**Izvod**

Screening testom je ispitana osetljivost 19 poljskih populacija krompirove zlatice (*Leptinotarsa decemlineata* Say.) prema abamektinu tokom 2008. (Futog, Čenej, Vilovo, Kovilj i Čurug), 2009. (Stepanovićevo, Gložane, Žabalj, Sirig, Šajkaš, Ljutovo, Šimuže, Bačko Gradište, Novo Miloševo i Kikinda) i 2012. godine (Zmajevo, Despotovo, Vilovo i Čurug). Pomenuti test je omogućio brzu procenu osetljivosti populacija prezimelih imaga krompirove zlatice prema abamektinu (preparat Abastate) koji je primenjen u preporučenoj (0.75 l/ha), dva (1.5 l/ha) i pet (3.75 l/ha) puta većim količinama od preporučene, metodom potapanja imaga u trajanju 5 sec. Smrtnost insekata je određena posle 72 h ekspozicije. Rezultati su korigovani za smrtnost u kontroli i prikazani preko efikasnosti. Prema IRAC (metoda br. 011), uz neznatnu korekciju, izvršena je procena osetljivosti krompirove zlatice prema skali 1-5 (1 - visoko osetljiva populacija ( $E=100\%$ ); 2 - osetljiva ( $100 > E \geq 95\%$ ); 3 - blago rezistentna ( $95 > E \geq 90\%$ ); 4 - rezistentna ( $90 > E \geq 50\%$ ); 5 - visoko rezistentna ( $E < 50\%$ )). Od pet ispitanih populacija krompirove zlatice u 2008. godini, prema količini za praktičnu primenu abamektina četiri su visoko osetljive ( $E=100\%$ ) i jedna osetljiva ( $E=97.5\%$ ). U 2009. godini od 10 ispitanih populacija, tri su blago rezistentne ( $E=92.6-94.6\%$ ), pet je rezistentno ( $E=64.6-88.7\%$ ) i dve su visoko rezistentne ( $E=22.5-48.3\%$ ) na količinu za praktičnu primenu abamektina. Od četiri ispitane populacije u 2012. godini jedna populacija je visoko osetljiva ( $E=100\%$ ), jedna osetljiva ( $E=95.8\%$ ) i dve su blago rezistentne ( $E=90.6-94.2\%$ ). Preživljavanje insekata primenom viših količina od onih za praktičnu primenu, dokaz je više da postoji deo populacije kod kojih je došlo do promene u osetljivosti ili selekcije na rezistentnost.

**Ključne reči:** screening test, krompirova zlatica, osetljivost, abamektin.

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