COMPARATIVE EVALUATION OF INSECTICIDES IN CONTROL OF *BOTHYNODERES PUNCTIVENTRIS* GERM. UNDER LABORATORY AND FIELD CONDITIONS

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ABSTRACT

Beet weevil *(Bothynoderes punctiventris* Germ.) is the most damaging pest of sugar beet in south-eastern Europe, from seedling emergence and in the first phases of crop development. Efficacy of insecticides (active ingredients of chlorpyriphos + bifenthrin, chlorpyriphos + beta-cyfluthrin and chlorpyriphos + cypermethrin) for the control of beet weevil was tested during 2010 and 2011 under laboratory and field conditions. A wet filter paper method (contact action) was employed in laboratory tests. Simultaneously, field trials were conducted (Čurug, Rimski Šančevi, Budisava, Kovilj) (contact and digestive action). The trial was designed according to EPPO method and insecticide efficacy was tested in accordance with the pest biology and phenophase of the crop. Insecticide efficacy was calculated using Abbot's formula, and damage of plants was assessed using 0-5 scale. Commercial insecticide Nurelle D (active ingredient of chlorpyriphos + cypermethrin) maintained high contact and digestive action on beet weevil, although it has been in use for 20 years. Commercial insecticide Pyrinex Super (active ingredient of chlorpyriphos + bifenthrin), although with reduced content of chlorpyriphos by 30% and of bifenthrin by 20%, achieved efficacy that was at the same level of significance with Nurelle-D, regardless of the experimental conditions (laboratory or field). Compared to Nurelle-D, the product MCW 784 (chlorpyriphos + beta-cyfluthrin) had significantly lower initial efficacy 3 h after application in laboratory trial, and also 24 h after application in field trial.

Key words: *Bothynoderes punctiventris*, efficacy, field, insecticide, laboratory.

INTRODUCTION

Bothynoderes punctiventris Germ. (Coleoptera: Curculionidae) is an oligophagous pest of sugar beet. It is harmful at adult stage, particularly when sowing is late and under conditions of high daily temperatures (Kereši, 2010). It is widely distributed in Asia and south-eastern Europe. The highest damages in Europe are recorded in Romania, Bulgaria, Serbia, Croatia, Hungary, Greece, Albania, Austria, Italy and Germany (Tanasijević and Simova-Tošić, 1987). In the XX[th] century over 2,000,000 ha of sugar beet were devastated by this pest in Eastern Europe. The highest damages in Serbia were registered in Vojvodina province, where it was the major cause of beet re-sowing in spring (Sekulić et al., 1997a), because one adult can destroy at least five one-day old sugar beet seedlings in 24 h (Čamprag et al., 2003). In the period from 1946 to 2000, according to Čamprag (2010) this pest destroyed young crops on over 180,000 ha in Vojvodina, which is the highest loss caused by a pest species on field crops in the XX[th] century. High attacks have been registered periodically (years: 1922-26, 1928-32, 1938-39, 1949-50, 1952-55, 1957-58, 1961-62, 1962-64, 1982-84 and 1993-1995 (Čamprag et al., 2000). In the mentioned years, the weevil was a major limiting factor in sugar beet production, and 90-100% of sugar beet crops, were treated with insecticides eight times, which brought production at limit of profitability (Sekulić et al., 1997b, 1997c).

In some regions of Serbia, an increase in population abundance of *B. punctiventris* has been registered in the last few years. The main causes are global climate changes, high temperatures, which have also been suggested for Ukraine by Fedorenko (2006), as well as

Received 07 January 2013; accepted 20 May 2013. First online: 10 April 2014. DII 2067-5720 RAR 2014-273

the privatization of social and state land in Serbia. In most cases agricultural land came in possession of people without professional reliance, who do not have practice, nor the scientific knowledge on sugar beet production technology. Improper crop rotation (sugar beet is being grown two to four years on the same field) and inadequate protection or cultural practices favour the increase in *B. punctiventris* abundance and higher damages. The above mentioned and the agricultural production oriented towards the highest profit, also caused reduction of acreage under this very important crop, and reduced the importance of sugar beet as raw material. In the period from 1981 to 1985 the average acreage under sugar beet cultivation in Vojvodina was 88,987 ha, and from 2001 to 2005, 53,727 ha, and the decreasing tendency continues.

The reduced importance of *B. punctiventris* control is also evident regarding the number of registered insecticides. In 2000, 38 products based on 10 active ingredients were registered for its control (Mitić, 2002), while in 2011, 13 products based on five active ingredients were registered for the same purpose (fenithrotion, fenthion, carbosulfan, chlorpyriphos + cypermethrin) (Sekulić and Jeličić, 2011).

High damages on sugar beet and difficulties in *B. punctiventris* control imposed a need for elaboration of a system of measures which would ensure optimal crop protection. The pest management system involves cultural, mechanical, chemical and biotechnical measures which are primarily based on long- and short-term forecast for this pest. By adequate and timely protection measures (spatial isolation, trapping canals, seed treatment with systemic insecticides, crop treatments) and good organization of the production, desired plant stand per ha can be retained. Chemical control is the main control measure (Inđić et al., 1998) and it will probably remain this way in the near future.

Intensive use of insecticides altered sensitivity of beet weevil populations, especially towards products which have been in use for a long period (Inđić et al., 2001; Vuković et al., 2006). For this reason, a need for strategy regarding insecticide use and new methods for sustainable control is necessary. The discovery and employment of aggregation attractant for beet weevil enables development and implementation of new methods for population abundance monitoring, but also for the control by adults mass trapping (Toth et al., 2005; Sivčev et al., 2006).

The use of product mixtures (with different modes of action) can be justified from biological, ecological and economical point of view, because it improves insecticide efficacy, reduces content of individual components in a mixture, consumption per area unit and prevents occurrence of resistance. Compatibility of compounds in a mixture was tested in the trial with carbosulphan and lambda-cyhalothrin, where carbosulphan provided persistence and lambda-cyhalothrin expressed high initial toxicity to beet weevil. By mixing active ingredients at defined ratio, some additive effect was achieved, indicating the need for finding an optimal ratio between components in the mixture (Inđić et al., 1992). Given the above mentioned, as well as the circumstance that in periods with low population abundance, problems related to this pest are neglected, and that next mass occurrence in most cases is faced unprepared, the aim of the paper was to test the efficacy of several insecticides (active ingredients of chlorpyriphos + bifenthrin, chlorpyriphos + beta-cyfluthrin and chlorpyriphos + cypermethrin) against *B. punctiventris* by comparative studies under field and laboratory conditions.

MATERIAL AND METHODS

Experiments were carried out in 2010 and 2011 under laboratory and field conditions.

Test insects. *B. punctiventris* adults were collected in April 2010 from Crvenka and in 2011 from Kula. At both localities, sugar beet was grown in two consecutive years on the same fields, and population abundance was very high. Collected adults were kept in wooden frame entomological cages with mesh sides (26x26x28 cm) in climatic chamber at temperature 5-9 °C until they were used in the test, without additional feeding during storage and without previous contact with insecticides. Before the trial was set up, adults were acclimatized at temperature of 23-25º C, for 3-4 h at laboratory conditions.

Insecticides. Insecticides used in the trials, ratio between active ingredients in products, as well application rates per unit area are given in Table 1.

Table 1. Insecticides and their application rates used for sugar beet weevil control

Insecticide ($g L^{-1}$ a.i.)	Product name	Formulation	Application rate ($L ha^{-1}$)*	Amount of a.i. ($g ha^{-1}$)
Chlorpyriphos (400) + bifenthrin (20)	Pyrinex Super 420	EC	1.5 2.0	600 + 30 800 + 40
Chlorpyriphos (250) + beta-cyfluthrin (12)	MCW 784	ZW	1 1.25	250 + 12 281.2 + 16
Chlorpyriphos (500) + cypermethrin (50)	Nurelle-D	EC	2.0	1000 + 100

*200 $l ha^{-1}$ of water; a.i. - active ingredient.

Laboratory trial. Contact action of insecticides on adults was tested using a wet filter paper method (Vuković, 2003; Vuković et al., 2003; Inđić and Vuković, 2012). Filter paper was placed in plastic boxes (20 × 14 × 5 cm), completely covering the bottom, and moistened with 5 mL of insecticide per box with micro applicator. Distilled water was the control. Ten beet weevil adults (sex ratio 1:1) were introduced in each box 1 h after pesticide application, when the paper was dry. One box represented one replicate and each application rate of tested insecticides was set in four replicates. Mortality of adults was assessed 3, 24 and 48 h after the insecticide application.

Criterion for insects mortality was a sum of dead (with no signs of life) + paralysed (not able to move or in abnormal position) adults. The test was conducted at 23-25 ºC, at relative air humidity of 40-45% and light regime of 16:8 h (light: dark).

Field trials were conducted according to OEPP methods for experimental design and data processing (EPPO 2006; Inđić and Vuković, 2012).

There is no available standard method for evaluation of biological efficacy of insecticides for sugar beet weevil. In test for contact-digestive action Nurelle-D (chlorpyriphos + cypermethrin) served as a standard. Sugar beet (cultivar Drena) at Čurug and Rimski Šančevi localities was sown on 5th and 10th of May 2010, respectively. In 2011 sowing of the same cultivar at Budisva and Kovilj localities was carried out on 8th and 10th of April. Inter-row space was 60 cm and in the row the distance was 2 cm. Insecticides were applied at the most susceptible phase of sugar beet (BBCH 10 - fully developed cotyledon leaves) when the highest damages caused by beet weevil occur. Plot size was 2 × 25 m (50 m^2), and the trial was set up in randomised block design with four replicates. Insecticides were applied with hand sprayer. After crop treatment with insecticides and deposit drying, within each replicate, five metal rings (R=40 cm) with tight mesh on the top were placed on the ground to cover 10 treated plants (excessive plants were removed). In each metal ring 10 vital adults were inserted. Mortality (dead + paralysed) of insects was assessed 24 and 48 h after insecticide application.

In 2010 and 2011 average daily temperatures during the experiment ranged from 20.6 to 22.3 ºC and from 16.3 to 19.7 ºC, respectively. On the third day from application, in each year, precipitation of 4.4 and 0.8 mm were registered, respectively. 24 and 48 h after insects were put in metal rings, the assessment of plant damage level (d) was carried out according to scale 0-5 (0 - no damage; 1 – d ≤ 10%; 2 – > 10 d ≥ 25%; 3 – > 25 d ≤ 50%; 4 – > 50 d ≤ 75%; 5 – > 75 d ≤ 100% /completely devastated plant/).

Insecticide efficacy was calculated using Abbott's formula (Wentzel, 1963) and significance of differences between treatments was analysed by Duncan's multiple range test, for confidence interval of 95%.

RESULTS

Specificity of sugar beet weevil as a pest, particularly at high population density and in the phase of seedlings emergence and cotyledon development (a critical period for its control) makes choice of insecticides very demanding. Primarily, insecticides should have high initial toxicity, i.e. cause rapid paralysis of insects and cessation of feeding.

The results of laboratory and field trials, conducted in 2010, on the efficacy of insecticides (chlorpyriphos + bifenthrin and chlorpyriphos + cypermethrin) for sugar beet weevil are presented in Table 2 and Figure 1. Under laboratory conditions, after 3 h of exposure, insecticides efficacy was high (97.5-100%) and at the same level of significance (F=0.5, P>0.05), regardless of active ingredients in products and applied rates. After adults were exposed to insecticides for 24 h, regardless the product, efficacy was 100%, and did not change, i.e. no revival of insects, even after 48 h. No significant differences in efficacy of tested insecticides depending on the exposure time were recorded, i.e. all tested insecticides exhibited high initial toxicity to *B. punctiventris* adults under laboratory conditions, regardless of active ingredients and their content in the products. In field trials (at Čurug), efficacy of chlorpyriphos + bifenthrin (1.5 L ha^{-1}) 24 h after insecticide application was 90%, which was significantly lower compared to the same product applied at rate of 2.0 L ha^{-1} and of chlorpyriphos + cypermethrin (2.0 L ha^{-1}). However, after 48 h, there was no significant difference in efficacy (95-100%) of all tested insecticides.

Table 2. Comparative efficacy (%) of insecticides for Bothynoderes punctiventris adults in laboratory and field tests in 2010

Site	Exposure (h)	Efficacy (%)			
		Chlorpyriphos + bifenthrin (1.5 L ha^{-1})	Chlorpyriphos + bifenthrin (2 L ha^{-1})	Chlorpyriphos + cypermethrin (2 L ha^{-1})	F value
Laboratory trial	3	97.5±5.00 aA	97.5±5.00 aA	100.0±0.00 aA	0.50NS
	24	100.0±0.00 aA	100.0±0.00 aA	100,0±0.00 aA	0.00NS
	48	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	0.00NS
F value		1.00NS	1.00NS	0.00NS	
Field test at Čurug	24	90.0±8.16 bA	100.0±0.00 aA	100.0±0.00 aA	6.00*
	48	95.0±10.00 aA	100.0±0.00 aA	100.0±0.00 aA	1.00NS
t value		0.775NS	0.00NS	0.00NS	
Field test at R. Šančevi	24	82.5±7.5 aA	90.0±8.16 aA	87.5± .57 aA	0.457NS
	48	87.5±5.0 aA	97.5±5.00 aA	97.5±5.00 aA	1.455NS
t value		0.471NS	1.567NS	1.82NS	

±SD values for differences among insecticide treatments; F values-Duncan's multiple range test; values with the same small letter are on the same level of significance regarding insecticide treatments; values with the same capital letter are on the same level of significance regarding exposure; NS P>0.05; *P< 0.05.

At the same locality, the average damage of sugar beet plants 24 h after the treatment ranged from 8.6 to 11.5% (Figure 1) while in the control it was 47.9%, which is significantly higher than in treated plots. 48 h after insecticides application, plant damage ranged from 9.4 to 11.5%, regardless of products and the rates applied, and it was 55.2% in the control, which is significantly higher than in treated plots. After 24 h from insecticides application, according to the observed damages, the adults stopped feeding, i.e. damages remained at the same level. At Rimski Šančevi locality, insecticides efficacy 24 h after the application ranged from 82.5 to 90% (F=0.457, P>0.05), and after 48 h from 87.5 to 97.5%, regardless of insecticides and application rates, which were at the same level of significance (F=1.455, P>0.05). Plant damage 24 h after insecticides application ranged from 12.1 to 20.8%, and 100% in the control. Similar results were also recorded 48 h after insecticides application.

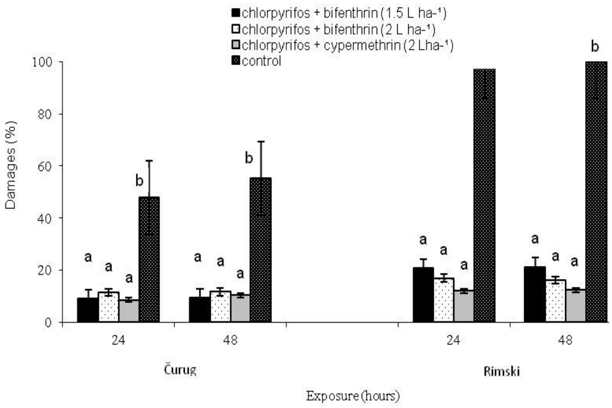

Figure 1. Damage (%) of sugar beet plants caused by sugar beet weevil in field test conducted in 2010

Insecticides efficacy (chlorpyriphos + beta-cyfluthrin and chlorpyriphos + cypermethrin) for *B. punctiventris* adults under laboratory and field trials conducted in 2011 is presented in Table 3 and Figure 2. In laboratory tests, efficacy of chlorpyriphos + beta-cyfluthrin (1 L ha^{-1}) after 3 h of adults exposure was significantly lower compared to the same product with application rate of 1.2 L ha^{-1}, and also to chlorpyriphos + cypermethrin (F=6.78*, P<0.05). After 24 and 48 h, efficacy was 100%, regardless of products, application rates and exposure time. After 3 h of exposure efficacy of chlorpyriphos + beta-cifluthrin, regardless of application rates (1.0 and 1.2 L ha^{-1}), was significantly lower than those after 24 and 48 h of exposure, while efficacy of chlorpyriphos + cypermethrin was high, regardless of exposure time. At Budisava locality chlorpyriphos + beta-cyfluthrin exhibited significantly lower efficacy (87.5%) compared to chlorpyriphos + cypermethrin (97.5%), regardless of application rates (F=5.33*, P<0.05). Depending on exposure time (24 and 48 h) no significant increase in insecticide efficacy was recorded in treated plots. Plant damage 24 h after insecticides application ranged from 4.0 to 5.6%, while in the control it was 18.9%, which is significantly higher than in treated plots. Significant increase of plant damage after 48 h was recorded in the control (53.7%), while in treated plots the damages after 24 h were reduced (3.7-4.6%), which can be explained by plants growth i.e. by increase in leaf mass (Figure 2).

Table 3. Comparative efficacy (%) of insecticides for *Bothynoderes punctiventris* adults in laboratory and field tests in 2011

Site	Exposure (h)	Efficacy (%)			
		chlorpyriphos+ beta-cyfluthrin (1.5 L ha^{-1})	chlorpyriphos+beta-cyfluthrin (2 L ha^{-1})	chlorpyriphos+ cypermethrin (2 L ha^{-1})	F value
Laboratory trial	3	72.0±12.58 bB	85.0±5.77 a B	96.0±5.77 aA	6.78*
	24	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	0.00NS
	48	100.0±0.00 aA	100.0±0.00 aA	100.0±0.00 aA	0.00NS
F value		19.11**	27.00**	3.00NS	
Field test at Budisava	24	87.5±5.00 bA	87.5±5.00 bA	97.5±5.00 aA	5.33*
	48	92.5±5.00 aA	95.0±5.77 a A	7.5 ± 5.00 aA	0.90NS
t value		1.41NS	1.96NS	0.00NS	
Field test at Kovilj	24	87.5±9.57 bA	85.0±5.77 bA	97.5±5.00 aA	5.11*
	48	95.0±10.0 a A	92.5±5.00 a A	100.0±0.00 aA	1.40NS
t value		1.08NS	1.96NS	1.00NS	

±SD values for differences among insecticide treatments; F values-Duncan's multiple range test; values with the same small letter are on the same level of significance regarding insecticide treatments; values with the same capital letter are on the same level of significance regarding exposure; NS P>0.05; *P<0.05; **P<0.01.

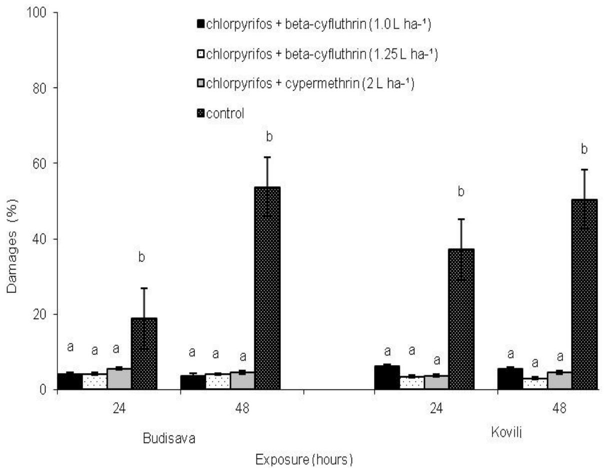

Figure 2. Damage (%) of sugar beet plants caused by sugar beet weevil in field test conducted in 2011

Insecticides efficacy at Kovilj locality was very similar to that at Budisava, whereas chlorpyriphos + beta-cifluthrin, regardless of application rates, had significantly lower efficacy than chlorpyriphos + cypermethrin (F=5.11*, $P<0.05$). Increased exposure time did not result in significant increase in insecticides efficacy. Plant damage 24 h after application ranged from 3.5 to 6.1% (Figure 2) and 37.2% in the control while after 48 h it was almost 50% in the control and ranged from 3.0 to 5.4% in treated plots.

DISCUSSION

Substantial damages on sugar beet, caused by sugar beet weevil, resulted in urgent need for development of a system of measures which would provide satisfying protection of the crop. The system involves cultural, mechanical, chemical and biotechnical measures, long- and short-term insect monitoring and forecast, as well as the strategy for insecticide application (Toth et al., 2005; Sivčev et al., 2006). Under production conditions in Serbia, management of this pest is recommended when 0.1-0.3 adults per m^2 are detected (Sekulić et al., 1995).

Populations of sugar beet weevil used in the tests originated from localities where sugar beet was cultivated for two consecutive years, which is intolerable in production technology of sugar beet (Vuković et al., 2011), and caused high abundance of this pest.

According to Inđić et al. (1997), differences in speed of action between organophosphorous insecticides depending on locality are noted. Therefore, monocrotophos acted 3.2-fold slower on population from Rimski Šančevi than on population from Bačka Topola. Differences in average lethal time (LT_{50}) have also been reported. In sugar beet weevil population from Subotica LT_{50} for cypermethrin, carbosulphan, metidathion and monocrotophos was 2, 4, 8 and 17 h, which points to 8-fold higher speed of action of cypermethrin than of monocrotophos (Inđić et al., 2000, 2001). Knowledge on speed of action of insecticides is of great importance for sugar beet weevil control, particularly when sugar beet is at early developmental stage, at the most susceptible phase.

Many authors mentioned binary mixtures of insecticides as strategic measure in sugar beet weevil control. High positive correlation between application rate of product and insect mortality was recorded for the mixture of carbosulphan + lambda-cyhalothrin applied against *B. punctiventris* (Inđić et al., 1992). According to Inđić et al. (1998) mixtures of carbamates and pyrethroids (carbofuran+ bifenthrin and carbosulphan+zeta-cypermethrin) expressed additive effect, when active ingredients in the mixture were applied at ratio ½ + ½ of field application rates. Vuković (2003) and Vuković et al. (2003) documented an increase in toxicity of organophosphates to sugar beet weevil with temperature increase (a positive temperature coefficient). An additive toxic effect of monocrotophos + cypermethrin mixture, affected by temperature (14, 20 and 25 ºC) was also quoted by the same authors (Vuković et al., 2006). Kereši et al. (2006) reported that no damage was caused by *B. punctiventris* after the application of imidacloprid + beta-cyfluthrin, while after the application of imidacloprid + tefluthrin the damage was 94%, i.e. 100% in the control.

Regarding the above mentioned and considering that the results of field and laboratory trials do not necessarily coincide, first laboratory tests under controlled conditions were conducted, and afterwards the field trials. Therefore, the discussion should refer to the applied insecticides and formulations, content of active ingredient per L of the product and application rates per ha, as well as simultaneously achieved results.

Nurelle-D (chlorpyriphos 500 g L^{-1} + cypermethrin 50 g L^{-1}) was registered 20 years ago (Mitić, 1992) but it is still highly efficient in *B. punctiventris* control. It is formulated as EC and applied at rate of 2.0 L ha^{-1}, or 1000 g of chlorpyriphos + 100 g of cypermethrin ha^{-1}. Pyrinex Super 420 EC is also formulated as EC (chlorpyriphos 400 g L^{-1} + bifenthrin 20 g L^{-1}), i.e. 600-800 g of chlorpyriphos + 30-40 g bifenthrin ha^{-1} and is registered for the same purpose since 2011. Product MCW 784, is formulated as ZW, and is a mixture of capsule suspension (CS) and emulsion oil in water (EW) (FAO and WHO 2006). It is applied at rates from 250 to 281.2 g of chlorpyriphos + 12-16 g of beta-cyfluthrin ha^{-1}. The differences in amounts of active ingredients (chlorpyriphos and pyrethroid) in the products are evident regarding application rate per hectare, which is probably due to high initial effect at their higher content. However, chlorpyriphos is included in EU Directive (Directive 2008/105/EC of the European Parlament 2008) and its residues in water and soil are monitored, and therefore reduction of application rates per ha or substitution with other, less dangerous substances is possible in future. Prescribed application rate of Pyrinex Super 420 EC per hectare contains 20-40% less chlorpyriphos compared to Nurelle-D. However achieved efficacy regardless of products, exposure time and conditions (laboratory, field) were at the same level of significance. The only significant decrease was recorded after 24 h of exposure and at application rate of 1.5 L ha^{-1} at Čurug locality in 2010. Experimental conditions in field should also be mentioned i.e. average daily temperatures during field trial were 20.0-22.3 ºC, while damage level in the control after 48 h was 55.2% at Čurug locality and 100% at Rimski Šančevi. In the product MCW 784 chlorpyriphos content was reduced to ¼ i.e. ⅓ compared to Nurelle-D. Also, this product is formulated as ZW (mix of CS and EW) and the results of laboratory tests after 3 h pointed to significantly lower efficacy compared to standard, as well as to significantly lower initial activity. Under field conditions at both localities, efficacy of both rates of the product after 24 h of exposure was significantly lower compared to standard. Hence, besides reduced contents of active ingredient, formulation type could also be the reason for slower action. This fact was presented by FAO and WHO (2006). Experimental conditions in 2011 were characterized by somewhat lower temperatures 16.3-19.7 ºC and therefore the level of plant damage in the control after 48 h was lower (50.5-53.7%) than in 2010, given that feeding of this pest is reduced at lower temperatures. This is also in accordance with the results presented by Skulić et al. (1997b).

CONCLUSIONS

Based on the experimental results on comparative efficacy of insecticides for the control of sugar beet weevil in 2010 and 2011, it can be concluded that all tested insecticides (active ingredients of chlorpyriphos + bifenthrin, chlorpyriphos + beta-cyfluthrin and chlorpyriphos + cypermethrin) expressed high efficacy both in laboratory and field conditions. Applied insecticides provided significantly higher protection of sugar beet compared to the control.

Acknowledgement

The work was realized within the Project entitled Rational use of fertilizers and pesticides in function of agro ecosystem protection, quality and cost-effectiveness of field crop production based on perennial trial-funded by Provincial Secretariat for Science and Technological Development of Vojvodina Province.

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