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RESEARCH ARTICLE

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Dynamic of *Brassicogethes aeneus* (F.) (Coleoptera, Nitidulidae) populations in Serbia's downriver Danube section and their susceptibility to insecticides

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Abstract

This paper presents data on the dynamic of *Brassicogethes aeneus* populations in winter oilseed rape (OSR) in the downriver section of the Danube in Serbia, which were acquired by yellow water traps and a beating method. Their susceptibility to several insecticides of different classes (organophosphates, pyrethroids and neonicotinoids) (adult vial test and dipping test) was tested over two seasons (2009 and 2010). *B. aeneus* populations developing under the agroecological conditions that exist in Serbia were monitored to detect the moment of OSR infestation during its sensitive growth stages. Adults were counted, and the data revealed that they infest winter OSR crops during the stem elongation growth stage defined by Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie - BBCH (30-31), reaching a population peak at the green-yellow bud stage (BBCH 57-59), and exceeding the economic threshold, while the populations decreased substantially during the subsequent stage. Laboratory test results did not confirm any changes in *B. aeneus* susceptibility/resistance to pyrethroid, organophosphate and neonicotinoid insecticides. Migration of *B. aeneus* populations was monitored as a way of developing predictive models for estimation of infestation severity and the timing of pest outbreaks under agroecological conditions existing in Serbia. As resistance to pyrethroids has been confirmed in *B. aeneus* populations in many European countries, their susceptibility will be further monitored.

Additional keywords: pollen beetle; abundance; organophosphates; pyrethroids; neonicotinoids; toxicity.

Abbreviations used: AVT (adult vial test); BM (beating method); DT (dipping test); OEPP/EPPO (European and Mediterranean Plant Protection Organization/ Organisation Européenne et Méditerranéenne pour la Protection des Plantes); OSR (oilseed rape); YWT (yellow water traps).

Authors' contributions: PM and PK conceived and designed research, PM, GA and MPG conducted experiments. PM, PK, TP and GA contributed to the data analysis. All authors wrote, read and approved the final manuscript.

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Introduction

The pollen beetle, *Brassicogethes aeneus* (F.), syn. *Meligethes aeneus* (F.) (Coleoptera: Nitidulidae), is a major pest in oilseed rape (*Brassica napus* L.) (OSR) fields in most European countries (Alford *et al.*, 2003), including Serbia (Sekulić & Kereši, 2007; Milovanović *et al.*, 2013). In Serbia, SE of Europe, only winter OSR is grown commercially, mostly in its northern parts (Vojvodina Province) and in Central Serbia, the production focusing in the downriver zone of the Danube in Serbia. Winter OSR crops have been continually grown in the observed region over many years, and control measures (including biocontrol) have often included insecticides with different modes of action (organophosphates, pyrethroids and neonicotinoids) to control *B. aeneus*.

Yield loss assessments in Europe have been reported to reach 50-60% (Nilsson, 1987), or 80% in spring OSR in Denmark (Hansen, 2003, 2004), while official data for Serbia and its region are missing. Damage is greater in seasons with cold spring because such weather slows down and extends plant development during sensitive growth stages. Harmfulness of *B. aeneus* depends on their numbers on OSR inflorescences, the timing of their appearance with regard to bud development, the period of time elapsing from the moment of *B. aeneus* settling to OSR flowering, the variety-depending capacity of regeneration of OSR crops, the cultivation methods applied, and of the weather conditions. Extreme damage occurs when a spell of warm weather at the end of winter causes *B. aeneus* to appear earlier in OSR crops, which only start to form flowering buds at the time. When cold weather delays OSR flowering, fewer *B. aeneus* adults are able to cause considerably more damage than more are able to do in seasons with shorter OSR flowering periods. Similar findings were reported by Bergant *et al.* (2005) for the developmental dynamics of onion thrips *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae) in Slovenia.

The presence of *B. aeneus* adults in OSR crops is easy to confirm by inspecting of inflorescences because the glossy, black insects that are often covered with pollen dust are actively mobile and easy to spot, while yellow water traps or similar devices put up in fields are attractive to flying species. The importance of monitoring *B. aeneus* populations is associated with rational and environment-friendly control measures for the pest because, based on their abundance assessment and adult infestation level, it is possible with greater precision to evaluate damage that the pest may cause to the crop, as well as pesticide treatments required (Williams, 2004). Traditionally, control of *B. aeneus* in winter OSR is based on spring insecticide treatments, usually with pyrethroids. However, where populations are potentially exposed to intensive applications of insecticides with the same mode of action, such as pyrethroids, and treatments are often consecutive within season, there is a high level of concern regarding the increased potential for selection pressure, which leads to increasingly widespread resistance development and prevents insecticide products from remaining active against the pest.

Since the earliest reports on *B. aeneus* resistance to pyrethroids in France and Scandinavia (Decoin, 2002; Hansen, 2003, 2004, 2008), its spreading in recent years across Europe has caused some major pest control problems in OSR fields in many European countries, *e.g.* France (Ballanger *et al.*, 2007), UK (Richardson, 2008a), Denmark (Hansen, 2003, 2004, 2008; Kaiser *et al.*, 2018), Poland (Węgorek *et al.*, 2009; Philippou *et al.*, 2011), Germany (Heimbach *et al.*, 2006; Thieme *et al.*, 2008; Heimbach & Müller, 2013), Switzerland (Philippou *et al.*, 2011), Austria (Slater *et al.*, 2011), Sweden (Kazachkova, 2007) and Czech Republic (Stará & Kocourek, 2017). Based on initial evidence, resistance monitoring activities have been initiated in many EU countries (Zimmer & Nauen, 2011), while organizations such as the European Plant Protection Organization (OEPP/EPPO) and Insecticide Resistance Action Committee (IRAC) have developed methods for testing the susceptibility of *B. aeneus* to organophosphates, pyrethroids and neonicotinoides, namely the dipping test and adult vial test, respectively (IRAC, 2006, 2009a,b; Thieme *et al.*, 2008).

Considering that *B. aeneus* studies have been missing in Serbia and no data are available on its populations dynamic and susceptibility to insecticides, three locations were chosen in the Danube downriver zone in order to create an appropriate pest management programme. The present study was design: 1) to determine the populations dynamic of *B. aeneus* during the critical phases of winter OSR growth by applying two sampling methods, the yellow water traps and the beating method, and 2) to conduct laboratory tests on the collected populations in order to determine toxicity/susceptibility levels to selected insecticides that have different modes of action (organophosphates, pyrethroids and neonicotinoid), applying two types of tests, the adult vial test and the dipping test.

Material and methods

Populations dynamic

The dynamic of *B. aeneus* populations in OSR crops was examined based on the time of their settlement in crops and adult counts during the sensitive stages of OSR growth on three locations in Serbia's downriver zone of the Danube: Kovin (2009 - 44°69′ N, 20°88′ E; 2010 - 44°73′ N, 20°96′ E), Smederevo (2009 - 44°66′ N, 20°95′ E; 2010 - 44°67′ N, 20°97′ E) and Požarevac (2009 - 44°62′ N, 21°14′ E; 2010 - 44°62′ N, 21°14′ E) over two seasons (2009, 2010). Two different methods of counting *B. aeneus* were used: yellow water traps (YWT) (Moericke, 1951) and beating method (BM) (Williams *et al.*, 2003). These methods are two conventional procedures for sampling *B. aeneus* either for providing a basis for plant protection decisions or for scientific purposes (Metspalu *et al.*, 2015). The experiment was performed in a randomized complex block design with four replicates. The sampling was performed at 50 m distance at least from field margins. *B. aeneus* were sampled on a weakly basis, 11 times in total, from 15 March to 24 May during both years, at the OSR growth stages BBCH 30 to 80, *i.e.* the phenological growth stages identified in the BBCH identification keys for oilseed rape (Weber & Bleiholder, 1990; Lancashire *et al.*, 1991).

— Counting of *B. aeneus* caught by YWT: Four YWTs (Moerickes dishes) were installed at plant height in each experimental field. YWTs were cleaned after each sampling and filled with fresh water.

— Counting of *B. aeneus* caught by BM: The method consists of tapping three times the terminal flower of each of 10 randomly chosen plants over a white plastic tray to dislodge the *B. aeneus* from buds and flowers and then the adults were counted. Samples were taken between 07:00 and 09:00 h, *i.e.* at the time of lower daily temperature when insects are less active.

Meteorological data

Meteorological data were collected from an automated weather station (*i*-Methos type) located in Smederevo. The variables used were: mean temperature (°C) and total rainfall (mm) (Fig. 1).

Susceptibility testing

Collection of B. aeneus

Susceptibility of the *B. aeneus* to insecticides was monitored on the three selected locations. Kovin, Smederevo and Požarevac represent the main growing regions of winter OSR in the downriver part of the Danube Basin in Serbia. Local populations were sampled from fields in those locations during each experimental year (2009, 2010). A total of 1500 specimens per population of adult *B. aeneus* were collected using the sweep net during April and May. They were transferred to the laboratory in plastic containers on dry filter paper. OSR flowers were added to each container for the *B. aeneus* to feed. Before bioassays were set up, *B. aeneus* adults were stored at +5 °C. Only adults that had good fitness were used in the bioassays.

Insecticides

Three populations of *B. aeneus* adults, collected in winter OSR crops in May of 2009 and 2010, were tested for susceptibility to insecticides showed in Table 1. Laboratory trials involved two standardized methods: adult vial test (AVT), recommended by IRAC (IRAC, 2006, 2009a,b), and dipping test (DT) (Thieme *et al.*, 2008), recommended by the OEPP/EPPO.

Susceptibility determinated by AVT

Vials with 48 cm^2 internal surface (5.5 cm × 2.5 cm) were used in these trials. The vials were filled with 500 µL water solution of each insecticide at test dosage, and then rotated until the deposits dried on internal walls. After draying, ten *B. aeneus* adults were added to each treated or untreated vial. Each test dosage and insecticide was represented in four replicates. Control vials contained only water. Trial conditions included: 22±2°C temperature, 60±5% RH and 16:8 LD photoperiod. Dead *B. aeneus* adults were counted after 1, 5 and 24 h of exposure to insecticide deposits. Assessment involved shaking insects out onto the middle of a circular paper of 15 cm diameter, and classifying the insects into one of two categories: (1) dead or immobile adults (those unable to walk out of the circle for one minute), and (2) live adults with visible mobility of legs, antennae etc.

Susceptibility determinated by DT

Unopened OSR flower buds were dipped into water solution of each insecticide for 5 sec. The flower

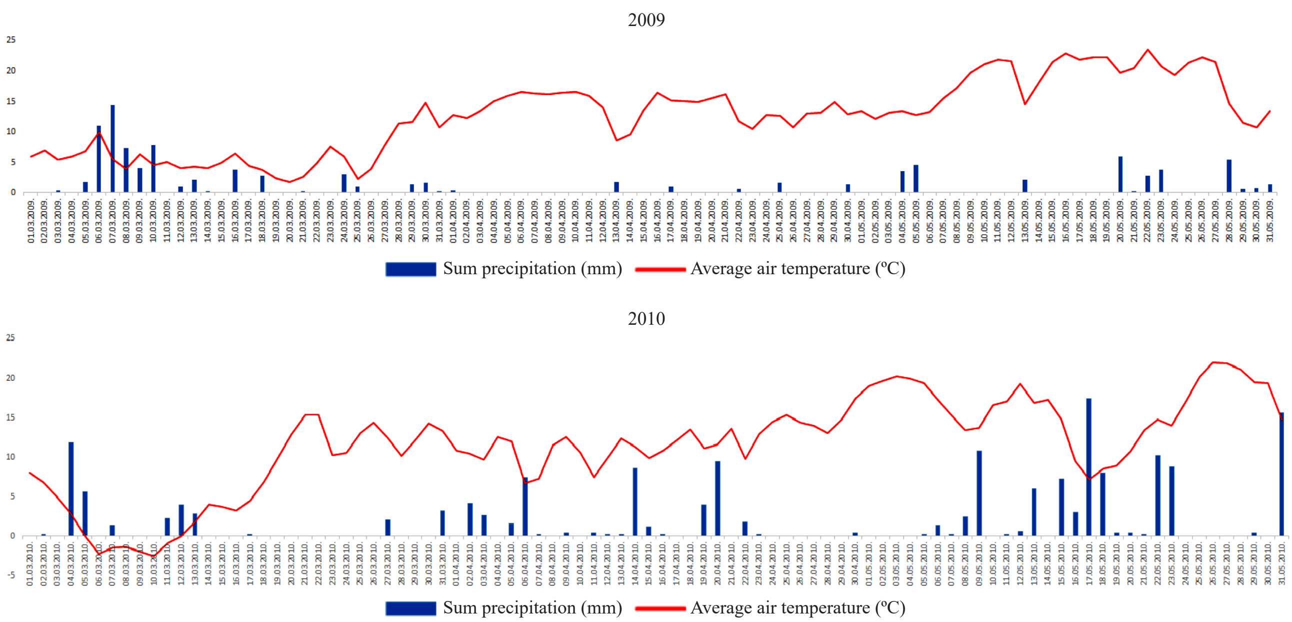

Figure 1. Meteo data for the period 1st March – 31th May of 2009 (up) and 2010 (down) obtained from the weather station Smederevo.

Table 1. Insecticides and concentrations applied.

Insecticide	a.i. content (g/L)	Test concentrations[a]					
		100% RLD[a]	75%	50%	25%	10%	5%
Lambda-cyhalothrin	25	0.075 [b]	0.056	0.0375	0.0187	0.0075	0.00375
		0.025 [c]	0.0187	0.0125	0.0062	0.0025	0.00125
Alpha-cypermethrin	100	0.1 [b]	0.075	0.05	0.025	0.01	0.005
		0.033 [c]	0.025	0.0167	0.0083	0.0033	0.00167
Bifenthrin	100	0.15 [b]	0.1125	0.075	0.0375	0.015	0.0075
		0.05 [c]	0.0375	0.025	0.0125	0.005	0.0025
Pirimiphos-methyl	500	5.0 [b]	3.75	2.5	1.25	0.5	0.25
		1.65 [c]	1.24	0.825	0.4125	0.165	0.0825
Chlorpyriphos + Cypermethrin	550	5.5 [b]	4.125	2.75	1.375	0.55	0.275
		1.815 [c]	1.361	0.9075	0.454	0.1815	0.09075
Thiacloprid	480	0.48 [b]	0.36	0.24	0.12	0.048	0.024
		0.1584 [c]	0.1188	0.0792	0.0396	0.0158	0.00792

[a]RLD= recommended label dose: [b]µg/cm² for adult vial test (AVT) and [c]mg/L for dipping test (DT). All data in horizontal lines for each insecticide.

buds were then left to dry on filter paper at 22±2°C temperature for 45 min. Glass vials of 48 cm² internal surface (5.5 cm × 2.5 cm) were filled with 10 flower buds and 10 *B. aeneus* adults each. The vials were then topped and left in upright position, under 22±2°C temperature, 60±5% RH and 16:8 h photoperiod. The trials were set up in four replicates, representing each test insecticide and each concentration. Dead and surviving adults of *B. aeneus* were counted after 1, 5 and 24 h of exposure. The vials were shaken briefly before inspection to check the difference between live and dead adults. Adults showing no mobility were noted as dead.

Data analysis

Populations dynamic

Before analysis, data on *B. aeneus* adults counts were transformed using *square root (x*+0.1*)*. The data were analyzed by a 4-way analysis of variance (ANOVA) in which the number of *B. aeneus* adults was the response variable, while the location, method, year and growth stage were main effects. The significance of mean differences was determined by Tukey-Kramer (HSD) test at p=0.05 (Sokal & Rohlf, 1995). Untransformed means of *B. aeneus* adult counts with standard deviations are shown in the figures. All data were processed in StatSoft version 7.1 (StatSoft Inc., Tulsa, Oklahoma).

Susceptibility testing

The trials testing *B. aeneus* susceptibility to test insecticides produced mortality percentages, which were corrected against control data using Abbott's formula to enable sound interpretation and comparison, while Probit analysis was used to derive the LC_{50} and LC_{95}, and the *lc-p* lines (Finney, 1971). Statistical significance of differences in toxicity indicators for the insecticides investigated was assessed based on the overlapping/non-overlapping of confidence intervals.

Results

Populations dynamic

All main effects and their interactions with the counts of *B. aeneus* adults were significant except the interaction location × year, which was not significant at the p=0.05 (Table 2).

— Counts of *B. aeneus* caught in YWTs: The emergence of overwintered *B. aeneus* adults in OSR crops was observed when OSR was at the growth stage of stem elongation (BBCH 30-31), *i.e.* on March 22, 2009 in all test locations (Kovin, Smederevo and Požarevac); on March 22, 2010 on the locations Kovin and Smederevo, and on March 29, 2010 on Požarevac location (Fig. 2). The number of *B. aeneus* adults gradually grew, reaching a peak during April (at the stage BBCH 51-59), and the maximum occurred on all locations on April 26 in both years.

In 2009 and 2010, the greatest number of *B. aeneus* was caught in Kovin, significantly more than in the other locations Smederevo and Požarevac.

— Counts of *B. aeneus* caught by BM: The first overwintered adults in winter OSR crops were observed when OSR was at the BBCH 30-31 growth stage (stem elongation): on March 15, 2009 on the locations Kovin and Smederevo, and on March 22 in Požarevac; on

Table 2. ANOVA parameters for main effects and their associated interactions based on *B. aeneus* counts in oilseed rape (OSR). Total df = 396.

Main effects	df	*F*	*p*
Locality	2	703.2	<0.01
Method	1	19761.9	<0.01
Year	1	410.5	<0.01
Growth stage	10	1218.3	<0.01
Locality × method	2	445.4	<0.01
Locality × year	2	2.3	0.11
Method × year	1	196.0	<0.01
Locality × growth stage	20	19.8	<0.01
Method × growth stage	10	869.0	<0.01
Year × growth stage	10	20.3	<0.01
Locality × method × year	2	6.1	<0.01
Locality × method × growth stage	20	18.6	<0.01
Locality × year × growth stage	20	3.4	<0.01
Method × year × growth stage	10	25.2	<0.01
Locality × method × year × growth stage	20	3.5	<0.01

March 22, 2010 on the location Kovin, and on March 29 on Smederevo and Požarevac locations (Fig. 2). The number of *B. aeneus* specimens gradually grew during April (BBCH 51-59), and populations reached peaks during the green-yellow bud stage (BBCH 57-59), reaching maximum on April 26. The period of flower bud formation was longer in 2009 (29 days), when the highest *B. aeneus* counts were recorded.

In 2009, the number of *B. aeneus* was highest in Kovin location and it differed significantly from the number collected on the locations Smederevo and Požarevac, and there was no difference between the letter two. In 2010, the number of *B. aeneus* caught on all three localities (Kovin, Smederevo and Požarevac) was similar for most of ratings.

The dynamic of *B. aeneus* populations was not found to differ significantly depending on the sampling methods, *i.e.* YWTs or BM.

Susceptibility testing

Data on the toxicity of test insecticides on *B. aeneus* populations are shown as the calculated parameters LC_{50} and LC_{95} determined by AVT (Tables 3 & 4) and by DT (Tables 5 & 6), and their corresponding *lc-p* lines for lambda-cyhalothrin (Figs. 3 & 4) and thiacloprid (Figs. 5 & 6) only as the *lc-p* lines of all variants of the other insecticides indicated overlapping of confidence intervals.

Susceptibility determined by AVT

— *B. aeneus* collected in 2009. Table 3 shows that, after 1 h of exposure, the most prominent differences between lambda-cyhalothrin as the most toxic

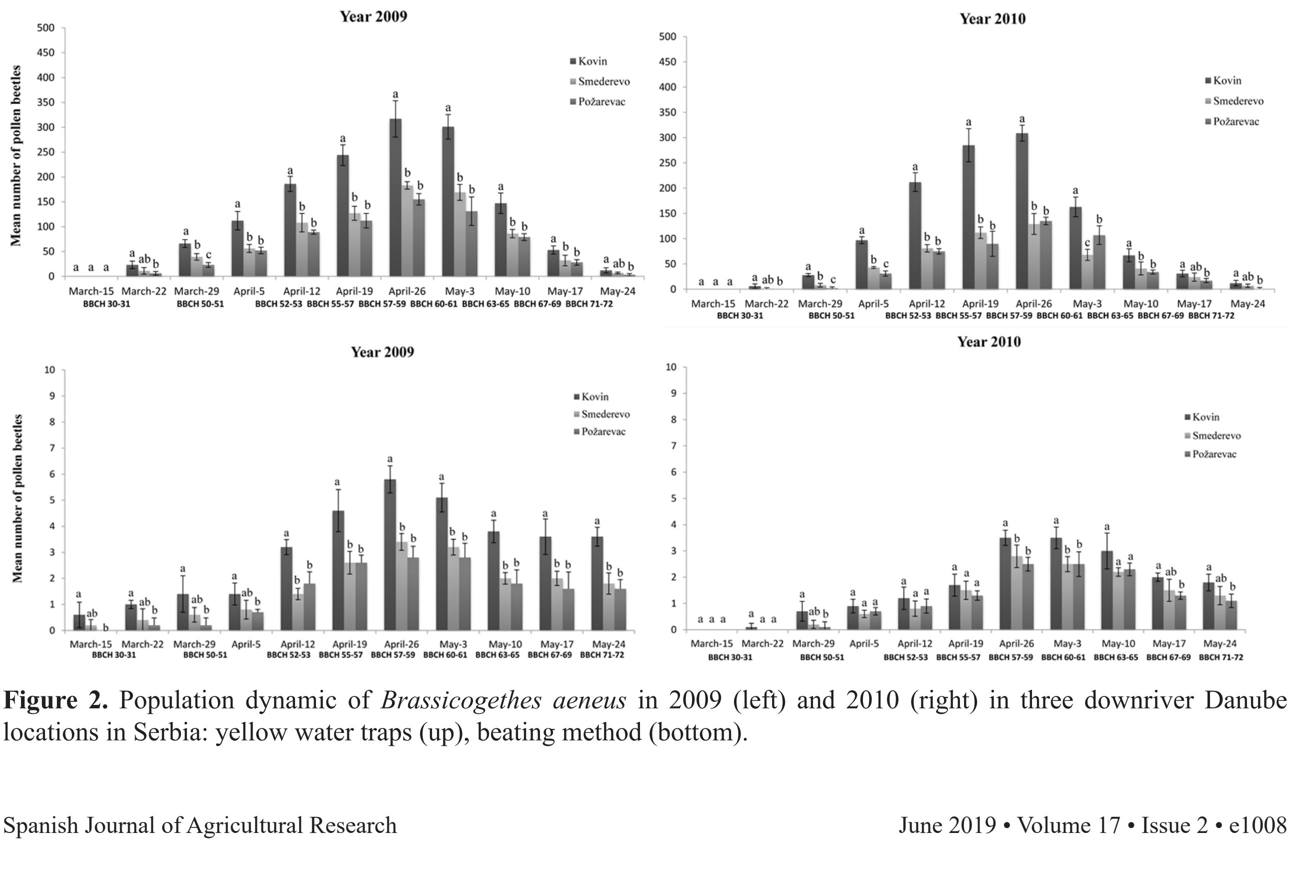

Figure 2. Population dynamic of *Brassicogethes aeneus* in 2009 (left) and 2010 (right) in three downriver Danube locations in Serbia: yellow water traps (up), beating method (bottom).

Table 3. Toxicity of insecticides to *B. aeneus* in 2009 using the adult vial test (AVT) in three downriver Danube locations in Serbia.

Insecticides	After (hours)	Kovin			Smederevo			Požarevac		
		LC_{50} (µg/cm²)[a] FL (0.05)[b]	LC_{95} (µg/cm²) FL (0.05)	Slope of *lc-p* line (± SE)	LC_{50} (µg/cm²) FL (0.05)	LC_{95} (µg/cm²) FL (0.05)	Slope of *lc-p* line (± SE)	LC_{50} (µg/cm²) FL (0.05)	LC_{95} (µg/cm²) FL (0.05)	Slope of *lc-p* line (± SE)
Lambda-cyhalothrin	1	0.011 (0.008-0.018)	0.44 (0.14-4.52)	1.03 ± 0.19	0.007 (0.005-0.010)	0.13 (0.06-0.45)	1.31 ± 0.19	0.011 (0.008-0.013)	0.069 (0.05-0.11)	2.03 ± 0.23
	5	0.004 (0.003-0.005)	0.07 (0.04-0.22)	1.31 ± 0.20	0.003 (0.002-0.004)	0.03 (0.02-0.05)	1.88 ± 0.23	0.003 (0.002-0.005)	0.034 (0.022-0.075)	1.63 ± 0.29
	24	0.002 (0.001-0.018)	0.02 (0.01-0.05)	1.46 ± 0.22	0.002 (0.001-0.003)	0.02 (0.01-0.03)	1.79 ± 0.24	0.002 (0.0005-0.003)	0.026 (0.017-0.068)	1.46 ± 0.32
Alpha-cypermethrin	1	0.019 (0.014-0.029)	0.30 (0.14-1.30)	1.37 ± 0.21	0.010 (0.008-0.013)	0.09 (0.05-0.17)	1.78 ± 0.22	0.015 (0.012-0.018)	0.07 (0.05-0.10)	2.52 ± 0.26
	5	0.005 (0.003-0.006)	0.06 (0.04-0.13)	1.50 ± 0.21	0.006 (0.005-0.008)	0.06 (0.04-0.13)	1.68 ± 0.22	0.007 (0.005-0.009)	0.04 (0.03-0.07)	2.24 ± 0.32
	24	0.003 (0.002-0.004)	0.03 (0.02-0.07)	1.60 ± 0.26	0.003 (0.002-0.004)	0.04 (0.03-0.09)	1.48 ± 0.22	0.004 (0.002-0.006)	0.04 (0.02-0.08)	1.79 ± 0.32
Bifenthrin	1	0.019 (0.014-0.025)	0.26 (0.14-0.80)	1.43 ± 0.20	0.024 (0.019-0.031)	0.20 (0.12-0.48)	1.78 ± 0.23	0.017 (0.011-0.024)	0.30 (0.17-0.77)	1.33 ± 0.20
	5	0.010 (0.008-0.014)	0.12 (0.07-0.27)	1.56 ± 0.20	0.008 (0.006-0.010)	0.07 (0.05-0.14)	1.71 ± 0.22	0.010 (0.006-0.014)	0.11 (0.07-0.22)	1.55 ± 0.23
	24	0.005 (0.002-0.011)	0.06 (0.02-0.21)	1.63 ± 0.34	0.004 (0.002-0.005)	0.04 (0.03-0.10)	1.61 ± 0.26	0.006 (0.003-0.010)	0.07 (0.05-0.18)	1.52 ± 0.29
Pirimiphos-methyl	1	0.33 (0.23- 0.44)	5.43 (2.96-15.27)	1.35 ± 0.19	0.34 (0.24-0.45)	5.72 (3.08-16.58)	1.34 ± 0.19	0.17 (0.06-0.27)	1.96 (1.26-4.63)	1.54 ± 0.31
	5	0.08 (0.04-0.12)	0.63 (0.40-1.54)	1.88 ± 0.38	0.04 (0.01-0.07)	0.35 (0.23-1.01)	1.79 ± 0.47	0.12 (0.03-0.21)	1.21 (0.77-3.59)	1.66 ± 0.43
	24	0.03 (0.01-0.07)	0.52 (0.30-2.63)	1.36 ± 0.40	0.03 (0.0001-0.06)	0.17 (0.12-1.43)	2.14 ± 0.89	0.09 (0.007-0.18)	0.75 (0.49-2.37)	1.79 ± 0.57
Chlorpyriphos + cypermethrin	1	0.62 (0.44-0.90)	17.70 (7.11-103.36)	1.12 ± 0.19	0.30 (0.22-0.40)	2.87 (1.85-5.75)	1.69 ± 0.22	0.24 (0.12-0.36)	2.49 (1.62-5.49)	1.63 ± 0.29
	5	0.05 (0.01-0.08)	0.50 (0.32-1.50)	1.69 ± 0.42	0.03 (0.01-0.06)	0.31 (0.19-1.33)	1.51 ± 0.51	0.15 (0.05-0.24)	1.14 (0.76-2.96)	1.86 ± 0.47
	24	0.05 (0.01-0.08)	0.25 (0.17-0.79)	2.46 ± 0.78	0.04 (0.002-0.07)	0.14 (0.11-1.72)	3.23 ± 1.47	0.16 (0.03-0.24)	0.68 (0.49-2.00)	2.65 ± 0.85
Thiacloprid	1	0.18 (0.11-0.50)	9.59 (2.00-425.59)	0.95 ± 0.20	0.14 (0.11-0.17)	1.06 (0.70-2.02)	1.84 ± 0.22	0.06 (0.04-0.07)	0.59 (0.38-1.14)	1.62 ± 0.21
	5	0.07 (0.05-0.10)	1.17 (0.52-5.61)	1.33 ± 0.23	0.075 (0.056-0.096)	0.67 (0.44-1.23)	1.74 ± 0.21	0.02 (0.01-0.04)	0.43 (0.24-1.40)	1.28 ± 0.25
	24	0.03 (0.02-0.04)	1.06 (0.36-14.02)	1.06 ± 0.23	0.044 (0.030-0.059)	0.37 (0.26-0.67)	1.78 ± 0.23	0.019 (0.008-0.03)	0.25 (0.15-0.62)	1.48 ± 0.29

[a]LC= lethal concentration; [b]FL= fiducial limit.

insecticide and chlorpyrifos+cypermethrin as the least toxic was found in Smederevo population, 48.6- and 63.5-fold regarding the LC_{50} and LC_{95}, respectively, while corresponding data were detected in the Požarevac population after 5 and 24 h exposure, *i.e.* 50.0- and 35.6-fold, and 80.0- and 28.8- fold at the LC_{50} and LC_{95}, respectively.

Lambda-cyhalothrin was the most toxic insecticide after all exposure intervals: after 1 h to Smederevo beetles at LC_{50} (0.007 µg/cm²) and Požarevac beetles at the LC_{95} (0.069 µg/cm²), after 5 h to Smederevo and Požarevac beetles at LC_{50} (0.003 µg/cm²) and LC_{95} (0.03 µg/cm²), respectively, and after 24 h to all tested populations. The least toxic after 1 h was chlorpyrifos+cypermethrin to Kovin beetles, while chlorpirifos+cypermethrin and pirimiphos-methyl were the least toxic after 5 h to Požarevac beetles at the LC_{50} (0.15 µg/cm²) and LC_{95} (1.21 µg/cm²), respectively, either without or with low-significant differences. After 24 h, the least toxic were chlorpyrifos+cypermethrin at the LC_{50} (0.16 µg/cm²) to Požarevac beetles and thiacloprid at the LC_{95} (1.06 µg/cm²) to Kovin beetles, either without or with low-significant differences.

— *B. aeneus* collected in 2010. Table 4 shows that the most prominent differences after all exposure intervals between lambda-cyhalothrin and alpha-cypermethrin

Table 4. Toxicity of insecticides to *B. aeneus* in 2010 using the adult vial test (AVT) in three downriver Danube locations in Serbia.

Insecticides	After (hours)	Kovin			Smederevo			Požarevac		
		LC_{50} (µg/cm²) FL (0.05)	LC_{95} (µg/cm²) FL (0.05)	Slope of *lc-p* line (± SE)	LC_{50} (µg/cm²) FL (0.05)	LC_{95} (µg/cm²) FL (0.05)	Slope of *lc-p* line (± SE)	LC_{50} (µg/cm²) FL (0.05)	LC_{95} (µg/cm²) FL (0.05)	Slope of *lc-p* line (± SE)
Lambda-cyhalothrin	1	0.033 (0.026-0.042)	0.28 (0.17-0.64)	1.77 ± 0.23	0.007 (0.005-0.009)	0.08 (0.04-0.19)	1.57 ± 0.20	0.009 (0.006-0.011)	0.063 (0.043-0.11)	1.93 ± 0.23
	5	0.015 (0.011-0.019)	0.12 (0.08-0.21)	1.83 ± 0.22	0.005 (0.003-0.006)	0.04 (0.02-0.07)	1.85 ± 0.23	0.003 (0.001-0.005)	0.052 (0.030-0.15)	1.35 ± 0.26
	24	0.008 (0.006-0.010)	0.047 (0.034-0.078)	2.09 ± 0.25	0.002 (0.001-0.003)	0.02 (0.01-0.05)	1.63 ± 0.26	0.0021 (0.0006-0.0036)	0.030 (0.018-0.080)	1.42 ± 0.31
Alpha-cypermethrin	1	0.029 (0.023-0.035)	0,17 (0,12-0,29)	2.11 ± 0.23	0.020 (0.016-0.024)	0.12 (0.084-0.18)	2.14 ± 0.23	0.007 (0.005-0.009)	0.09 (0.05-0.22)	1.47 ± 0.20
	5	0.022 (0.018-0.028)	0,12 (0,09-0,19)	2.25 ± 0.25	0.0094 (0.0066-0.012)	0.076 (0.053-0.13)	1.81 ± 0.23	0.005 (0.003-0.006)	0.05 (0.03-0.10)	1.62 ± 0.22
	24	0.015 (0.012-0.019)	0,095 (0,068-0,15)	2.09 ± 0.24	0.006 (0.0037-0.0080)	0.042 (0.029-0.081)	1.93 ± 0.31	0.002 (0.001-0.003)	0.03 (0.02-0.06)	1.61 ± 0.26
Bifenthrin	1	0.020 (0.015-0.028)	0.35 (0.16-1.34)	1.32 ± 0.20	0.020 (0.015-0.025)	0.15 (0.10-0.29)	1.83 ± 0.23	0.043 (0.035-0.052)	0.21 (0.16-0.33)	2.36 ± 0.25
	5	0.011 (0.007-0.014)	0.16 (0.09-0.47)	1.40 ± 0.21	0.008 (0.005-0.012)	0.12 (0.075-0.25)	1.44 ± 0.22	0.027 (0.022-0.033)	0.12 (0.09-0.17)	2.62 ± 0.28
	24	0.005 (0.004-0.008)	0.16 (0.09-0.47)	1.58 ± 0.22	0.004 (0.0008-0.007)	0.091 (0.051-0.33)	1.19 ± 0.28	0.020 (0.016-0.024)	0.09 (0.07-0.14)	2.49 ± 0.29
Pirimiphos-methyl	1	0.52 (0.40-0.68)	6.07 (3.46-15.28)	1.53 ± 0.20	0.20 (0.09-0.31)	2.38 (1.51-5.63)	1.52 ± 0.29	0.275 (0.142-0.413)	4.16 (2.61-9.23)	1.39 ± 0.22
	5	0.11 (0.08-0.15)	0.70 (0.49-1.28)	2.09 ± 0.32	0.11 (0.021-0.21)	1.28 (0.81-4.20)	1.57 ± 0.42	0.11 (0.025-0.21)	1.43 (0.911-3.67)	1.49 ± 0.35
	24	0.09 (0.07-0.11)	0.30 (0.22-0.54)	3.25 ± 0.64	0.081 (0.0044-0.17)	0.87 (0.55-3.11)	1.60 ± 0.51	0.083 (0.005-0.17)	0.86 (0.55-3.00)	1.62 ± 0.51
Chlorpyriphos + cypermethrin	1	0.62 (0.42-0.93)	18.57 (7.00-141.37)	1.11 ± 0.21	0.38 (0.22-0.54)	5.01 (3.00-12.81)	1.47 ± 0.25	0.54 (0.37-0.72)	5.31 (3.54-9.99)	1.65 ± 0.21
	5	0.11 (0.06-0.15)	0.84 (0.56-1.69)	1.84 ± 0.30	0.16 (0.06-0.27)	1.67 (1.09-3.79)	1.63 ± 0.34	-	-	-
	24	0.06 (0.03-0.09)	0.29 (0.20-0.64)	2.54 ± 0.64	0.08 (0.02-0.18)	0.81 (0.52-2.90)	1.66 ± 0.56	-	-	-
Thiacloprid	1	0.24 (0.19-0.33)	2.48 (1.35-6.91)	1.63 ± 0.22	0.08 (0.06-0.12)	1.37 (0.62-5.76)	1.35 ± 0.21	0.19 (0.12-0.40)	3.98 (1.25-46.84)	1.24 ± 0.23
	5	0.16 (0.13-0.21)	1.47 (0.89-3.41)	1.71 ± 0.23	0.04 (0.03-0.05)	0.73 (0.36-2.73)	1.29 ± 0.21	0.09 (0.07-0.11)	0.51 (0.31-1.39)	2.14 ± 0.37
	24	0.12 (0.091-0.15)	1.03 (0.66-2.07)	1.75 ± 0.22	0.02 (0.01-0.03)	0.32 (0.18-0.90)	1.32 ± 0.21	0.04 (0.02-0.07)	0.34 (0.10-1.41)	1.78 ± 0.38

–: not possible to calculate.

as the most toxic, and chlorpyrifos+cypermethrin and thiacloprid as the least toxic insecticides were found at the LC_{50} and LC_{95} in Smederevo population, 54.3- and 62.6-fold, 32.0- and 41.8-fold, and 40.0- and 43.5-fold, respectively.

The most toxic insecticides after all exposure intervals were lambda-cyhalothrin and alpha-cypermethrin: after 1 h to beetles from Smederevo and Požarevac at the LC_{50} (0.007 µg/cm²) and LC_{95} (0.08 and 0.09 µg/cm², respectively), and after 5 h and 24 h to beetles from Požarevac. The least toxic after 1 h exposure were chlorpyrifos+cypermethrin to Kovin beetles, while thiacloprid was the least toxic to Kovin beetles after 5 and 24 h.

Susceptibility determined by DT

— *B. aeneus* collected in 2009. As shown in Table 5, after 1 h and 5 h exposure the most prominent differences between lambda-cyhalothrin and alpha-cypermethrin as the most toxic insecticides, and pirimiphos-methyl and chlorpyrifos+cypermethrin as the least toxic were found in the population originating from Požarevac, 59.0- and 65.1-fold at the LC_{50} and LC_{95}, respectively (after 1 h), and 23.3- and 19.7-fold at the LC_{50} and LC_{95}, respectively (after 5 h), while the greatest difference after 24 h was found in the Kovin population, 20.0- and 17.5-fold at the LC_{50} and LC_{95}, respectively.

Lambda-cyhalothrin and alpha-cipermethrin were the most toxic insecticides to Požarevac beetles at the

Table 5. Toxicity of insecticides to *B. aeneus* in 2009 using the dipping test (DT) in three downriver Danube locations in Serbia.

Insecticides	After (hours)	Kovin			Smederevo			Požarevac		
		LC_{50} (mg/L) FL (0.05)	LC_{95} (mg/L) FL (0.05)	Slope of *lc-p* line (± SE)	LC_{50} (mg/L) FL (0.05)	LC_{95} (mg/L) FL (0.05)	Slope of *lc-p* line (± SE)	LC_{50} (mg/L) FL (0.05)	LC_{95} (mg/L) FL (0.05)	Slope of *lc-p* line (± SE)
Lambda-cyhalothrin	1	0.018 (0.014-0.029)	0.11 (0.06-0.93)	2.03 ± 0.51	0.013 (0.009-0.020)	0.15 (0.06-0.88)	1.56 ± 0.32	0.010 (0.007-0.015)	0.10 (0.05-0.45)	1.69 ± 0.34
	5	0.013 (0.009-0.016)	0.05 (0.03-0.14)	3.01 ± 0.77	0.007 (0.005-0.010)	0.07 (0.04-0.26)	1.63 ± 0.31	0.010 (0.006-0.012)	0.03 (0.02-0.06)	3.29 ± 0.75
	24	0.004 (0.002-0.006)	0.04 (0.02-0.25)	1.62 ± 0.38	0.003 (0.002-0.004)	0.02 (0.01-0.05)	1.94 ± 0.33	0.004 (0.002-0.006)	0.02 (0.01-0.08)	2.01 ± 0.43
Alpha-cypermethrin	1	0.011 (0.009-0.016)	0.09 (0.05-0.28)	1.82 ± 0.31	0.015 (0.011-0.022)	0.14 (0.07-0.60)	1.69 ± 0.32	0.010 (0.008-0.013)	0.09 (0.05-0.17)	1.78 ± 0.22
	5	0.010 (0.007-0.013)	0.03 (0.02-0.04)	4.04 ± 0.86	0.005 (0.003-0.006)	0.04 (0.02-0.08)	1.90 ± 0.30	0.006 (0.005-0.008)	0.06 (0.04-0.13)	1.68 ± 0.22
	24	0.003 (0.001-0.005)	0.02 (0.01-0.11)	1.89 ± 0.47	0.002 (0.001-0.004)	0.014 (0.009-0.039)	2.29 ± 0.49	0.003 (0.002-0.004)	0.04 (0.03-0.09)	1.48 ± 0.22
Bifenthrin	1	0.019 (0.014-0.025)	0.26 (0.14-0.80)	1.43 ± 0.20	0.03 (0.02-0.05)	0.29 (0.13-2.00)	1.67 ± 0.36	0.03 (0.02-0.04)	0.28 (0.15-0.80)	1.63 ± 0.23
	5	0.010 (0.008-0.014)	0.12 (0.07-0.27)	1.56 ± 0.20	0.011 (0.008-0.015)	0.09 (0.05-0.23)	1.81 ± 0.31	0.008 (0.006-0.01)	0.10 (0.06-0.21)	1.54 ± 0.20
	24	0.005 (0.002-0.011)	0.06 (0.02-0.21)	1.63 ± 0.34	0.006 (0.003-0.009)	0.06 (0.03-0.26)	1.60 ± 0.34	0.004 (0.002-0.006)	0.05 (0.03-0.10)	1.59 ± 0.23
Pirimiphos-methyl	1	0.39 (0.25-0.55)	5.66 (2.81-22.34)	1.41 ± 0.25	0.39 (0.25-0.57)	7.21 (3.27-37.14)	1.30 ± 0.24	0.41 (0.27-0.57)	4.46 (2.42-14.61)	1.59 ± 0.28
	5	0.14 (0.10-0.18)	0.49 (0.34-1.03)	3.04 ± 0.62	0.06 (0.01-0.08)	0.24 (0.16-1.28)	2.60 ± 0.91	0.06 (0.005-0.08)	0.19 (0.3-1.52)	3.17 ± 1.26
	24	0.06 (0.01-0.09)	0.30 (0.19-3.04)	2.36 ± 0.88	-	-	-	-	-	-
Chlorpyriphos + cypermethrin	1	0.60 (0.44-0.78)	3.78 (2.38-8.78)	2.06 ± 0.34	0.56 (0.38-0.80)	7.84 (3.75-35.00)	1.43 ± 0.26	0.59 (0.40-0.83)	6.51 (3.31-26.15)	1.57 ± 0.29
	5	0.10 (0.05-0.15)	0.54 (0.35-1.57)	2.27 ± 0.55	0.09 (0.04-0.12)	0.38 (0.25-1.34)	2.63 ± 0.75	0.14 (0.09-0.21)	0.59 (0.34-2.77)	2.70 ± 0.74
	24	0.008 (0.03-0.12)	0.35 (0.23-1.70)	2.70 ± 0.87	-	-	-	0.01 (0.04-0.13)	0.34 (0.23-1.35)	3.02 ± 0.92
Thiacloprid	1	0.19 (0.12-0.40)	3.98 (1.25-46.84)	1.24 ± 0.23	0.08 (0.06-0.10)	0.45 (0.27-1.47)	2.14 ± 0.42	0.06 (0.05-0.08)	0.53 (0.32-1.15)	1.78 ± 0.22
	5	0.09 (0.07-0.11)	0.51 (0.31-1.39)	2.14 ± 0.37	0.05 (0.04-0.07)	0.16 (0.12-0.30)	3.48 ± 0.78	0.05 (0.04-0.06)	0.35 (0.23-0.67)	1.85 ± 0.22
	24	0.04 (0.02-0.07)	0.34 (0.10-1.41)	1.78 ± 0.38	0.02 (0.01-0.03)	0.14 (0.08-0.34)	2.21 ± 0.44	0.03 (0.02-0.04)	0.22 (0.16-0.38)	2.00 ± 0.22

–: not possible to calculate.

LC_{50} after 1 h exposure, while alpha-cypermethrin and lambda-cyhalothrin were the most toxic after 5 h and 24 h exposure of Smederevo beetles to their LC_{50} (0.005 and 0.002 mg/L, respectively), and Kovin and Požarevac beetles to the LC_{95} (0.03 and 0.02 mg/L, respectively). The least toxic after 1 h of exposure were chlorpyrifos+cypermethrin to beetles from Kovin at the LC_{50} (0.6 mg/L) and to beetles from Smederevo at the LC_{95} (7.84 mg/L), while pirimiphos-methyl was the least toxic after 5 h and 24 h exposure of Kovin beetles at the LC_{50} (0.14 and 0.06 mg/L, respectively) and chlorpyrifos+cypermethrin at the LC_{95} (0.59 and 0.35 mg/L) to Požarevac and Kovin beetles, respectively.

— *B. aeneus* collected in 2010. Table 6 shows that after 1 and 24 h exposure the most prominent differences between lambda-cyhalothrin and alpha-cypermethrin as the most toxic insecticides and chlorpyrifos+cypermethrin and thiacloprid as the least toxic was found in Smederevo population at the LC_{50} and LC_{95}, 62.0- and 177.0-fold, and 50.0- and 26.0-fold, respectively, while the corresponding data for Kovin population after 5 h exposure were 14.0- and 23.4-fold, respectively.

The most toxic insecticides after all exposure intervals were lambda-cyhalothrin and alpha-cipermethrin: after 1 h to Smederevo and Požarevac beetles

Table 6. Toxicity of insecticides to *B. aeneus* in 2010 using the dipping test (DT) in three downriver Danube locations in Serbia.

Insecticides	After (hours)	Kovin			Smederevo			Požarevac		
		LC_{50} (mg/L) FL (0.05)	LC_{95} (mg/L) FL (0.05)	Slope of *lc-p* line (± SE)	LC_{50} (mg/L) FL (0.05)	LC_{95} (mg/L) FL (0.05)	Slope of *lc-p* line (± SE)	LC_{50} (mg/L) FL (0.05)	LC_{95} (mg/L) FL (0.05)	Slope of *lc-p* line (± SE)
Lambda-cyhalothrin	1	0.016 (0.012-0.026)	0.27 (0.11-1.30)	1.37 ± 0.22	0.010 (0.008-0.013)	0.10 (0.06-0.24)	1.67 ± 0.22	0.011 (0.008-0.018)	0.44 (0.14-4.52)	1.03 ± 0.19
	5	0.005 (0.004-0.007)	0.05 (0.03-0.11)	1.65 ± 0.20	0.005 (0.004-0.008)	0.07 (0.04-0.16)	1.47 ± 0.20	0.004 (0.003-0.005)	0.07 (0.04-0.22)	1.31 ± 0.20
	24	0.003 (0.002-0.004)	0.03 (0.02-0.06)	1.58 ± 0.21	0.001 (0.0008-0.002)	0.02 (0.01-0.06)	1.36 ± 0.21	0.002 (0.001-0.003)	0.02 (0.01-0.05)	1.46 ± 0.22
Alpha-cypermethrin	1	0.015 (0.012-0.020)	0.13 (0.08-0.30)	-	0.015 (0.011-0.022)	0.28 (0.12-1.20)	1.30 ± 0.20	0.019 (0.014-0.029)	0.30 (0.14-1.30)	1.37 ± 0.21
	5	0.006 (0.003-0.012)	0.08 (0.02-0.42)	1.52 ± 0.35	0.004 (0.002-0.006)	0.13 (0.053-0.88)	1.10 ± 0.22	0.005 (0.003-0.006)	0.06 (0.04-0.13)	1.50 ± 0.21
	24	0.003 (0.001-0.009)	0.07 (0.01-0.56)	1.25 ± 0.34	0.003 (0.002-0.004)	0.03 (0.02-0.07)	1.48 ± 0.21	0.003 (0.002-0.004)	0.03 (0.02-0.07)	1.60 ± 0.26
Bifenthrin	1	0.03 (0.02-0.05)	0.31 (0.13-2.31)	1.68 ± 0.37	0.020 (0.015-0.028)	0.35 (0.16-1.34)	1.32 ± 0.20	0.018 (0.013-0.025)	0.16 (0.08-0.56)	1.76 ± 0.33
	5	0.02 (0.01-0.03)	0.19 (0.08-3.28)	1.64 ± 0.45	0.011 (0.007-0.014)	0.16 (0.09- 0.47)	1.40 ± 0.21	0.011 (0.007-0.017)	0.11 (0.06-0.44)	1.66 ± 0.34
	24	0.007 (0.004-0.011)	0.06 (0.03-0.24)	1.78 ± 0.39	0.005 (0.004-0.008)	0.06 (0.04-0.53)	1.58 ± 0.22	0.005 (0.002-0.008)	0.04 (0.03-0.11)	1.77 ± 0.35
Pirimiphos-methyl	1	0.52 (0.40-0.68)	6.07 (3.46-15.28)	1.53 ± 0.20	0.33 (0.23-0.44)	5.43 (2.96-15.27)	1.35 ± 0.19	0.31 (0.23-0.40)	3.54 (2.18-7.70)	1.55 ± 0.20
	5	0.11 (0.08-0.15)	0.70 (0.49-1.28)	2.09 ± 0.32	0.08 (0.04-0.12)	0.63 (0.40-1.54)	1.88 ± 0.38	0.019 (0.0001-0.048)	0.23 (0.14-0.97)	1.51 ± 0.54
	24	0.09 (0.07-0.11)	0.30 (0.22-0.54)	3.25 ± 0.64	0.03 (0.01-0.07)	0.52 (0.30-2.63)	1.36 ± 0.40	-	-	-
Chlorpyriphos + cypermethrin	1	0.41 (0.28-0.55)	8.49 (4.18-30.11)	1.24 ± 0.19	0.62 (0.44-0.90)	17.70 (7.11-103.36)	1.12 ± 0.19	0.40 (0.28-0.53)	6.80 (3.61-20.28)	1.33 ± 0.19
	5	0.03	0.16	2.46 ± 1.05	0.05 (0.01-0.08)	0.50 (0.32-1.50)	1.69 ± 0.42	0.01	0.13	-
	24	-	-	-	0.05 (0.01-0.08)	0.25 (0.17-0.79)	2.46 ± 0.70	-	-	-
Thiacloprid	1	0.18 (0.11-0.50)	9.59 (2.00-425.59)	0.95 ± 0.20	0.07 (0.04-0.10)	1.11 (0.45-8.09)	1.34 ± 0.27	0.08 (0.06-0.12)	1.37 (0.62-5.76)	1.35 ± 0.21
	5	0.07 (0.05-0.10)	1.17 (0.52-5.61)	1.33 ± 0.23	0.05 (0.03-0.07)	0.84 (0.35-6.40)	1.32 ± 0.28	0.04 (0.03-0.05)	0.73 (0.36-2.73)	1.29 ± 0.21
	24	0.03 (0.02-0.04)	1.06 (0.36-14.02)	1.06 ± 0.23	0.02 (0.01-0.03)	0.23 (0.14-0.67)	1.65 ± 0.30	0.02 (0.01-0.03)	0.32 (0.18-0.90)	1.32 ± 0.21

-: not possible to calculate.

at the LC_{50} (0.01 mg/L), and to Smederevo beetles at the LC_{95} (0.1 mg/L), after 5 h to Požarevac and Smederevo beetles at the LC_{50} (0.004 mg/L) and to Kovin and Požarevac beetles at the LC_{95} (0.05 mg/L), and after 24 h to Smederevo beetles at the LC_{50} (0.001 mg/L) and Kovin beetles at the LC_{95} (0.07 mg/L). The least toxic after 1 h was chlorpyrifos+cypermethrin to Smederevo beetles, after 5 h it was pirimiphos-methyl at the LC_{50} (0.11 mg/L) and thiacloprid at the LC_{95} (1.17 mg/L) to Kovin beetles, and after 24 h the least toxic were chlorpyrifos+cypermethrin at the LC_{50} (0.05 mg/L) to Smederevo beetles, and thiacloprid at the LC_{95} (1.06 mg/L) to Kovin beetles.

Generally, regarding all populations collected in 2009 and 2010, we can see that the position of all *lc-p* lines for lambda-cyhalothrin and thiacloprid (Tables 3 to 6 and Figs. 3 to 6) were clearer and more accuracy in the AVT type of testing than the DT. Also, the obtained slopes of *lc-p* lines for both insecticides show that lambda-cyhalothrin and thiacloprid were significantly more toxic to Požarevac beetles collected in 2009 after only 1 h of exposure than they were to the other two populations, whose *lc-p* lines mostly overlapped.

Discussion

B. aeneus emerged in winter OSR crops in the downriver stretch of the Danube in Serbia during

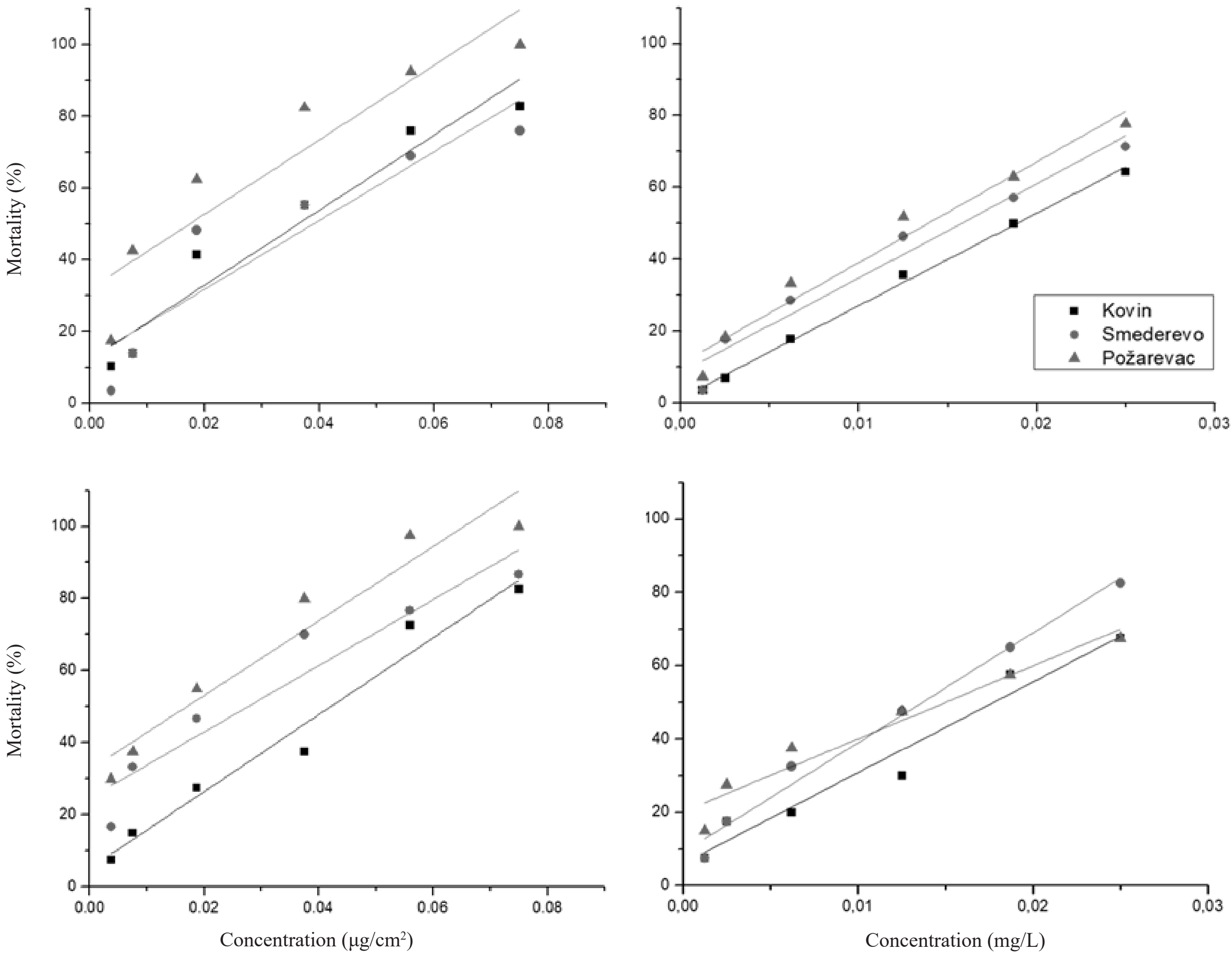

Figure 3. *lc-p* lines for lambda-cyhalothrin effects on *Brassicogethes aeneus* populations after 1 h of exposure, collected in 2009 (up) and 2010 (down). AVT, adult vial test (left) and DT, dipping test (right).

March when crops were at the stem elongation growth stage (BBCH 30-31) with maximum daily temperature exceeding 10°C, which was noted on March 12 2009, and March 22 2010. According to Láska & Kocourek (1991) first *B. aeneus* adults emerged when temperature exceeded 10.2°C, or at a later growth stage (BBCH 51-54) in the second half of March (Williams, 2006). The data show that emergence of *B. aeneus* adults depends on favourable temperature and that *B. aeneus* fly over from other early flowering plants into OSR crops when it exceeds 15°C (Alford *et al.*, 2003).

B. aeneus adult numbers continued to grow during the later OSR growth stage (BBCH 50-59), and their highest counts were noted at the green-yellow bud growth stage (BBCH 57-59). After that, in May (BBCH 61-69), their numbers decreased substantially. Similarly, other authors showed that *B. aeneus* adults were most numerous at bud extension and yellow bud stages and thereafter declined (Sedivy, 1993; Walter & Northing, 2007; Petraitiene *et al.*, 2008; Vaitelyte *et al.*, 2011; Metspalu *et al.*, 2015). A wider range of maximum *B. aeneus* abundance regarding OSR growth stages has been reported for stages from the late green bud until the beginning of flowering (BBCH 53-63), which occurs in April (Williams, 2006).

However, the BM is required for determining the threshold for timely and cost-efficient treatments regarding insect number. Insecticide applications therefore may be delayed for some 10 days after the conventional timing "before flowering" (Maceljski, 1999). In Serbia, other thresholds given by Maceljski are acceptable (Maceljski & Jelovčan, 2007). During the test years, threshold was exceeded on the test location during the sensitive growth stages of winter OSR, and it was from 2.8 to 5.8 adult/terminal flower in 2009, and from 2.5 to 3.5 adult/terminal flower in 2010. Thresholds in winter OSR crops in European countries are given either as numbers per terminal flower (Croatia, Slovakia; range 0.8-3 adult/terminal flower based on growth stage), or as numbers per plant (in Switzerland, Czech Republic, France, Denmark, Hungary, Latvia,

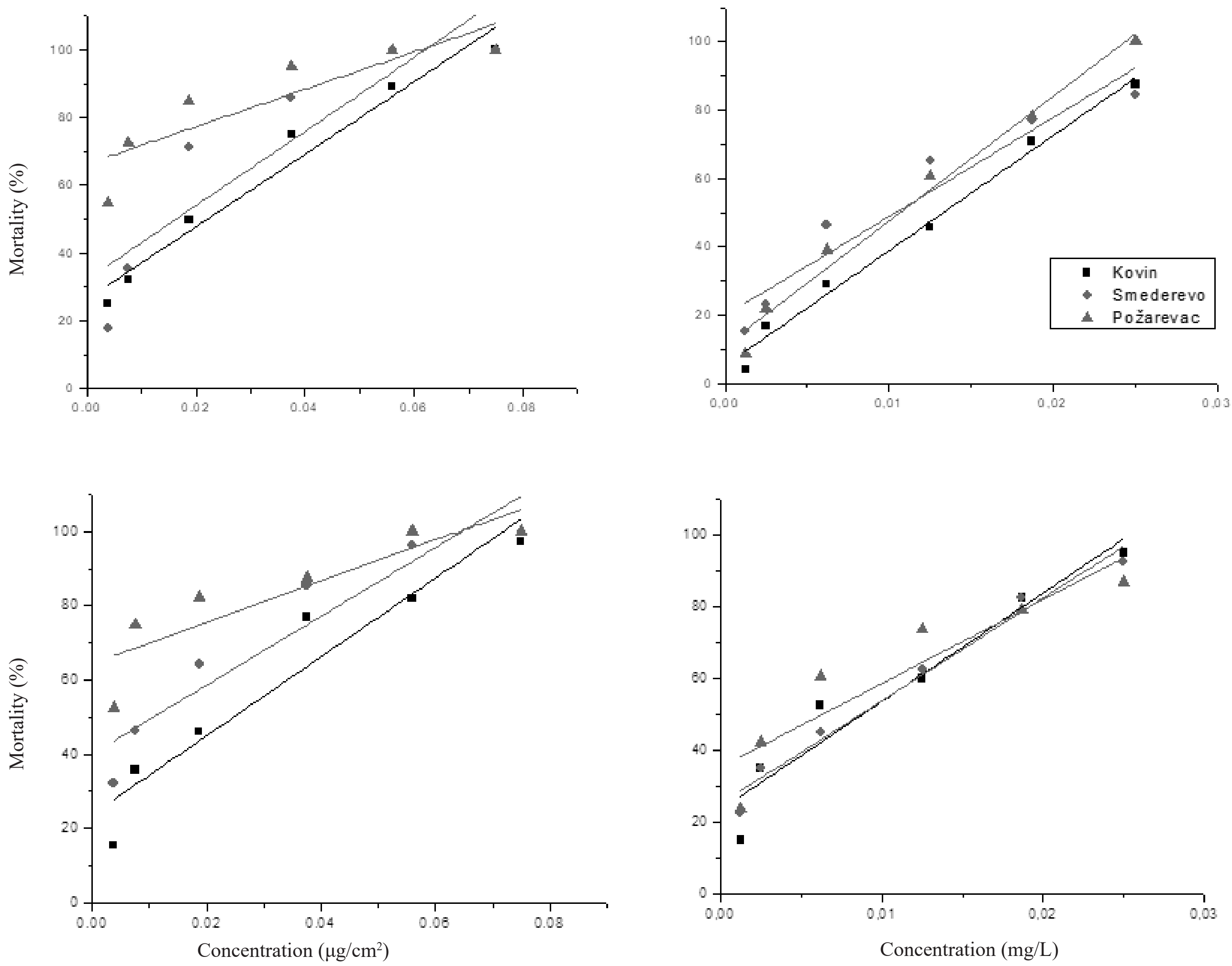

Figure 4. *lc-p* lines for lambda-cyhalothrin effects on *Brassicogethes aeneus* populations after 5 h of exposure, collected in 2009 (up) and 2010 (down). AVT, adult vial test (left) and DT, dipping test (right).

Luxemburg, Norway, Slovenia, Poland, Netherlands and Sweden) with numbers ranging from 1 to 6 per plant and commonly graduating according to growth stage (Richardson, 2008b). Critical thresholds in Germany and France are 3-4 adult /terminal flower at the BBCH 50-51 stage, 7-8 at the BBCH 52-53 stage, and more than 8 at the BBCH 55-59 stage (Williams, 2010).

Our results show that considerably more *B. aeneus* were found on the location Kovin than in Smederevo and Požarevac over both years of trials. Also, more *B. aeneus* were caught in 2009 than in 2010. In a similar study in Lithuania, the least *B. aeneus*, 2.5 adult/flower were caught in 2009, and the most in 2007, 18 adults/flower (Vaitelyte *et al.*, 2011). Differences in the abundance of *B. aeneus* over the years are common, and could be the result of several factors, *e.g.* differences in the rate of plant growth triggered by weather conditions, direct effects of temperature, humidity or plant species (Petraitiene *et al.*, 2008; Vaitelyte *et al.*, 2011). In Lithuania, OSR developed faster in seasons with minimum daily temperature exceeding 15°C, buds developed over a briefer time period of 15 days, and 3 adult/flower were counted; in years with maximum daily temperatures close to 15°C, when bud formation took a longer time (21-27 days), the counts reached as much as 7 adult/flower (Petraitiene *et al.*, 2008). In our present study, monitoring of the duration of bud development over the period 2009-2010 showed that this process was longer in 2009 (29 days), and showed a positive correlation with *B. aeneus* adult counts, compared with 2010. Harsh winter temperatures can also have harmful impact on overwintering *B. aeneus* and reduce the number of emerging *B. aeneus* in spring (Hokkanen, 2000).

The results of susceptibility testing obtained in this study based on the parameters LC_{50} and LC_{95} and appropriate *lc-p* lines (for lambda-cyhalothrin and thiacloprid) showed that all tested insecticides were highly toxic to all three tested populations of *B. aeneus* with toxicity increasing with the duration of *B. aeneus*

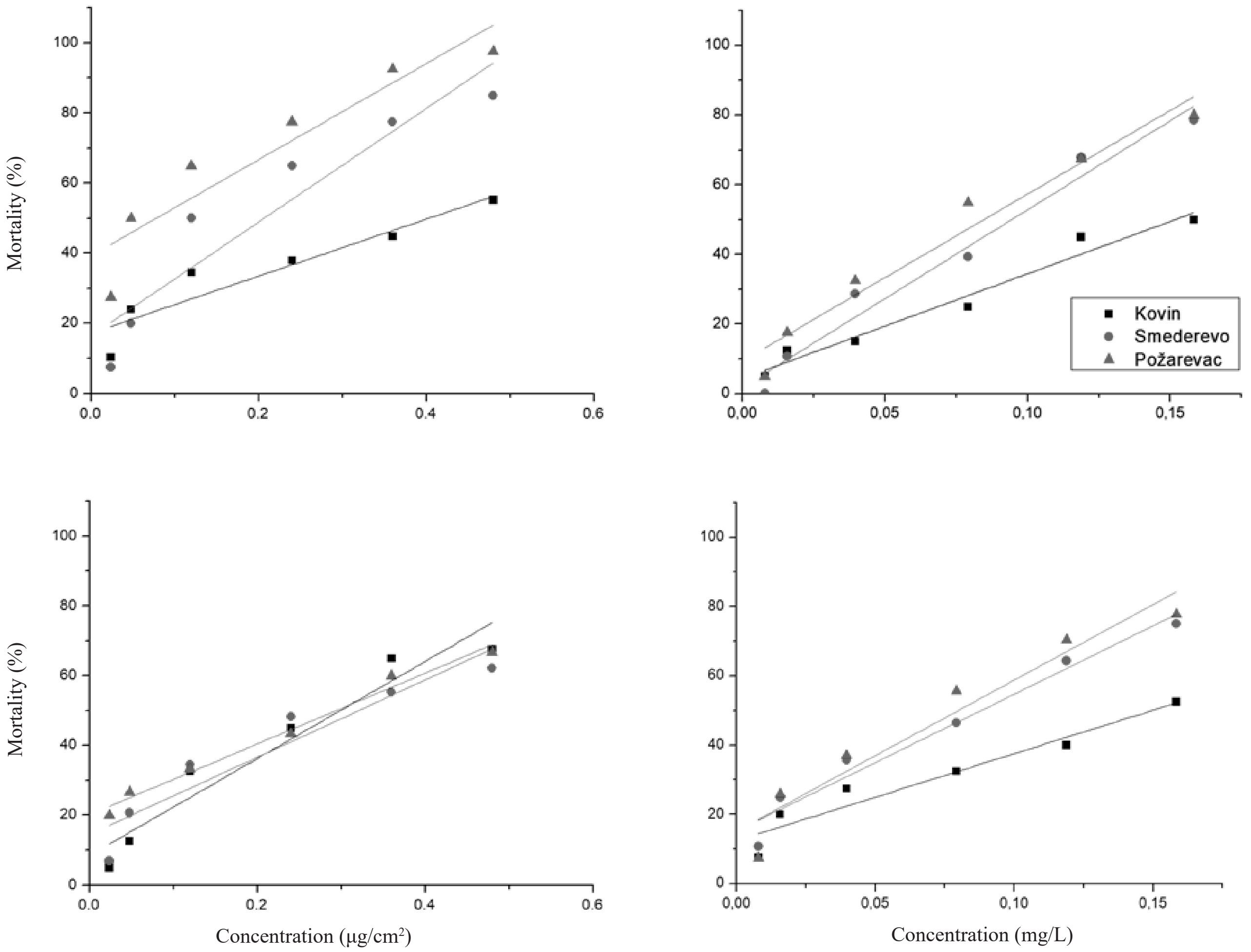

Figure 5. *lc-p* lines for thiacloprid effects on *Brassicogethes aeneus* populations after 1 h of exposure, collected in 2009 (up) and 2010 (down). AVT, adult vial test (left) and DT, dipping test (right).

exposure. Also, the *lc-p* lines, especially the slopes and overlapping/non-overlapping of confidence intervals, showed that the resulting insecticide toxicity changed either not at all or very low, depending on population/ location of *B. aeneus*, season in which they were sampled and tested, and the type of test (AVT and/or DT).

In AVTs, only bifenthrin and pirimiphos-methyl did not change their toxicity after exposing for 1 h all three test populations of *B. aeneus* collected in 2009 and 2010, and the same effect of lambda-cyhalothrin and alpha-cypermethrin was found regarding *B. aeneus* collected in 2009, and chlorpyrifos+cypertmethrin and thiacloprid as affecting *B. aeneus* collected in 2010. However, lambda-cyhalothrin was about 4-fold less toxic to *B. aeneus* from Kovin collected in 2010 than to populations from Smederevo and Požarevac, while alpha-cypermethrin applied to Požarevac population collected in 2010 and thiacloprid to those collected in 2009 were *c*. 3 times more toxic. After 5 and 24 h exposure, all test insecticides showed mostly unchanging level of toxicity to all three populations collected in 2009 and 2010 or a change only occur in *B. aeneus* collected in 2010 at the LC_{50} level, as decreasing of alpha-cypermethrin and bifenthrin toxicity for Kovin and Požarevac populations than for Smederevo and Požarevac populations, some 2.5- and 6-fold, and 4- and 3-fold, respectively. Also, the two-year AVT testing revealed that the recommended doses (showed in Table 1) and 75% of the recommended doses of all insecticides caused ≥ 95% mortality of *B. aeneus* in all populations, as well as some 50%, 25%, 10% and even 5% recommended doses. The only data exceeding the recommended dose (0.48 µg/cm^2) referred to thiacloprid treatment of Kovin population for 24 h at the LC_{95}, 1.06 µg/cm^2 in 2009 and 1.03 µg/cm^2 in 2010.

Regarding the DT type of testing, all test insecticides retained stable toxicity to all three populations collected in 2009 and 2010 after 1, 5 and 24 h of *B. aeneus* exposure, except pirimiphos-methyl, which was after 5 h exposure at the LC_{50} level 2 times less toxic to Kovin population than to population from Smederevo and Požarevac. The analysis of the two-year

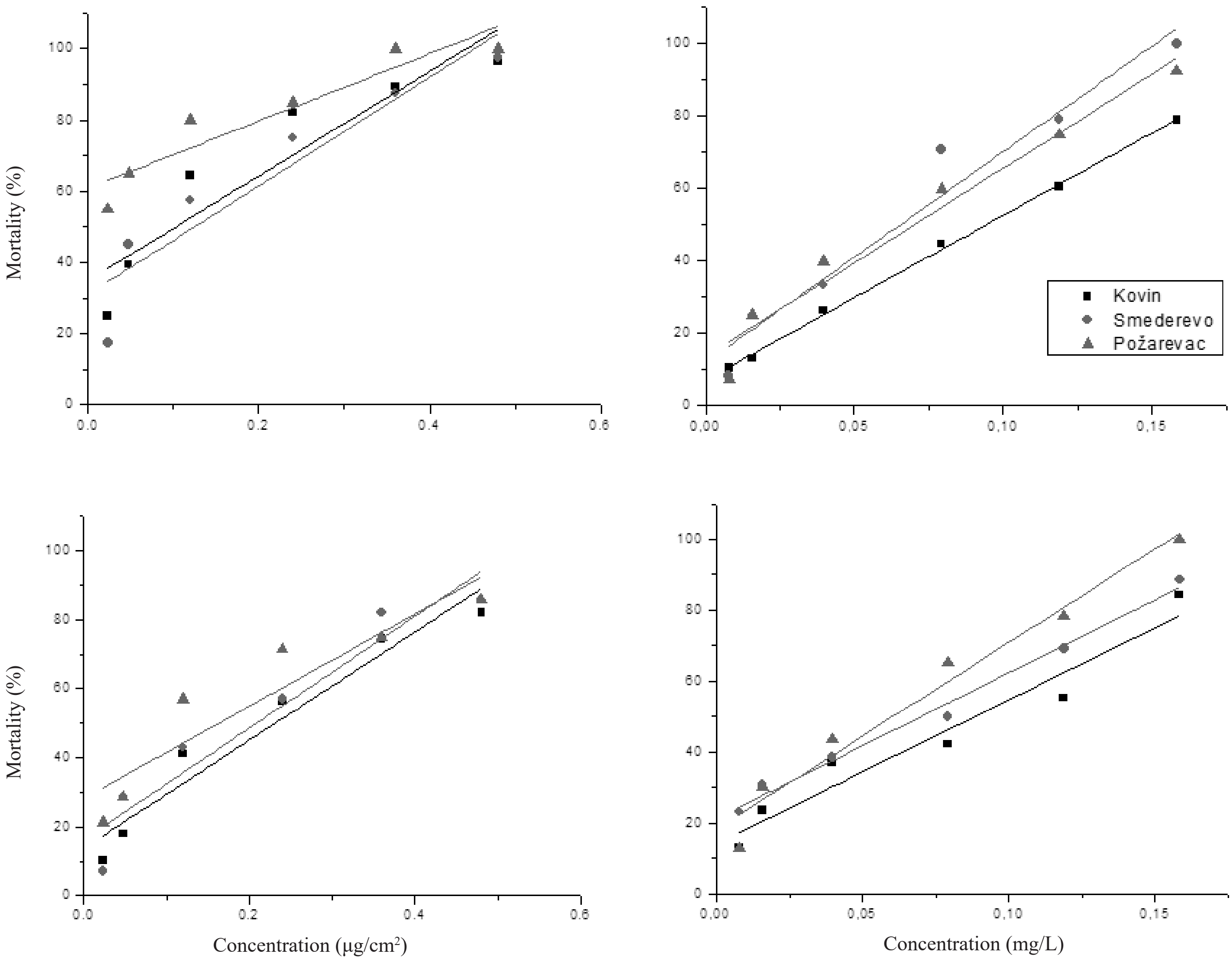

Figure 6. *lc-p* lines for thiacloprid effects on *Brassicogethes aeneus* populations after 5 h of exposure, collected in 2009 (up) and 2010 (down). AVT, adult vial test (left) and DT, dipping test (right).

results of DTs for all test populations showed that the recommended concentrations and 75% recommended concentrations of all insecticides, except thiacloprid, caused ≥ 95% mortality, even at 50%, 25%, 10% and 5% recommended concentrations. However, the LC_{95} data for Kovin population exposed for 24 h were higher than the recommended concentrations for: lambda-cyhalothrin 0.03 mg/L in 2010 and 0.04 mg/L in 2009, alpha-cypermethrin 0.07 mg/L in 2010, and bifenthrin 0.06 mg/L in 2009 and 2010. Concerning the population Smederevo, the data for 24 h exposure at the LC_{95} showed that they exceeded the recommended dose for bifenthrin 0.06 mg/L in 2009 and 2010, while in population from Požarevac the value for alpha-cypermethrin was 0.04 mg/L in 2009. Interestingly, thiacloprid achieved ≥ 95% *B. aeneus* mortality only to Smederevo population in 2009, while in all other trial variants after 24 hours of exposure at the LC_{95} it was higher than the recommended concentration (0.1584 mg/L), 0.22-1.06 mg/L.

Available literature has largely documented variable toxicity data for different classes of tested insecticides (with different modes of actions) to *B. aeneus* populations, and their variation in sensitivity under laboratory tests, which depends on population and year of study. Studies in Germany on the suscepbility of populations of *B. aeneus*, and some other insect pest species to the pyrethroids lambda-cyhalothrin and cypermethrin revealed a notable reduction in suscepbility only in *B. aeneus*, while it was lower in *C. napi* and *C. pallidactylus*. However, data aquired in laboratory testing may be assumed valid only when they are considered in the context of corresponding field data (Heimbach *et al.*, 2006). Therefore, special field trials Milovanović *et al.* (2013) were organized to assess the efficacy of those insecticides using OEPP/EPPO methodology on the same locations during three successive vegetation seasons in 2008, 2009 and 2010 at the winter rapeseed development stage of visible flower buds but still closed (BBCH 55-59) by counting the present *B. aeneus* adults. In 2009, three days after treatment, all tested insecticides achieved efficacy ≥ 92%, except thiacloprid at Kovin and Smederevo locations, while all insecticides showed significantly lower efficacy, 79-92%, at all three locations after seven days of treatment. In those intervals, the highest efficacy (≥ 90%) was demonstrated by pirimiphos-methyl and chlorpyrifos+cypermethrin, at all three locations, and by lambda-cyhalothrin and alpha-cypermethrin applied to Požarevac population. In 2010, no statistically significant differences were detected between the tested insecticides at the locations Smederevo and Požarevac three days after treatment, while only thiacloprid showed a significantly lower efficacy against Kovin population than the other insecticides. As in 2008 and 2009, the efficacy of the tested insecticides seven days after treatment was again lower than it was after three days, 76-87%.

The results of the testing of three *B. aeneus* populations at three downriver locations in the Serbian section of the Danube showed that the number of *B. aeneus* in winter OSR was highest during the green-yellow bud stage, exceeding the economic threshold. Considerably more *B. aeneus* were found in Kovin locality than in Smederevo and Požarevac, and in 2009 rather than in 2010. According to our susceptibility rating scheme, the populations tested in this experiment were found to belong to the second group, *i.e.* populations susceptible to the test insecticides, while *B. aeneus* from the location Požarevac were found as most susceptible, and Kovin population was least susceptible. Also, the data acquired by the AVT method proved more precise results. Lambda-cyhalothrin was found to be the most toxic insecticide in most trials, while thiacloprid was least toxic. However, as resistance of *B. aeneus* populations has been documented in many European countries, it is necessary to revaluate population susceptibility to lambda-cyhalothrin, thiacloprid and other insecticides in the same locations (especially Kovin) within the next ten years. Populations from other locations along the Danube should also be examined to provide a basis for creating a useful Insecticide Resistance Management (IRM) strategy and incorporating it into Integrated Pest Management program for *B. aeneus* control in Serbia and region.

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