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Scientific paper

Efficacy of Spinosad and Abamectin against Different Populations of Red Flour Beetle (*Tribolium castaneum* Herbst) in Treated Wheat Grain

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SUMMARY

The efficacy of spinosad and abamectin against *T. castaneum* adults from a laboratory population with normal susceptibility to contact insecticides and against malathion-resistant populations from Nikinci and Jakovo was tested in the laboratory (25±1°C and 60±5% r.h.). The insecticides were applied to 500 g of untreated wheat grain for each of the following application doses: 0.25, 0.5, 1.0, 2.5 and 5.0 mg a.i./kg. After treatment, wheat was divided into three equal subsamples and 50 *T. castaneum* adults from each of the three test populations were released the next day into jars for each dose. Mortality was evaluated after 7, 14 and 21 days of exposure to treated wheat grain.

Generally, higher concentrations and longer exposure periods resulted in higher efficacy of both insecticides, but abamectin was significantly more effective than spinosad against all three tested populations. After 7 days of exposure, mortality did not exceed 30% in any test variant. Fourteen days after treatment with the highest dose (5 mg/kg) of spinosad, mortality was highest (75%) in the laboratory population, while treatment with the same dose of abamectin achieved the highest mortality (58%) in the laboratory and Jakovo populations. After 21 days, spinosad applied at the rate of 5 mg/kg was most effective (97% mortality) in the laboratory population, while 88% efficacy was recorded in Jakovo population and 87% in Nikinci population. Abamectin doses of 2.5 and 5 mg/kg caused high adult mortality of 94-100% in the laboratory and Jakovo populations, and a significantly lower mortality in Nikinci population (75 and 86%, respectively).

Statistically significant differences in the efficacy of spinosad, and particularly of abamectin, were detected among the three tested populations, the greatest difference being between the laboratory and Nikinci populations, which clearly indicates that resistance of *T. castaneum* adults to malathion had a significant influence.

Keywords: Efficacy; Abamectin; Spinosad; Populations; *T. castaneum*

INTRODUCTION

Contact insecticides, alongside with fumigants, are still most widely used to control insects of stored products. They were introduced at the end of the 1960s, and organophosphates were the most frequently used group of these insecticides at the begining, while pyrethroids have been used more intensively in the latest period (White and Leesch, 1996; Kljajić, 2008; MacBean, 2012). However, selection pressure built over the decades-long use of organophosphates, particularly malathion, and pyrethroids, has resulted in a changed susceptibility or resistance of some storage insect populations to those insecticides (Subramanyam and Hagstrum, 1996; Kljajić and Perić, 2005; Boyer et al., 2012). As a result of that negative effect, their use is becoming increasingly limited, although they continue to be an important option in IPM programs for protection of stored products from insect pests (Zettler and Arthur, 2000). One of the ways to manage the problem of resistance of stored-product insects to different insecticides and to alleviate selection pressure in practice is to introduce new insecticides. The latest studies have shown that the neonicotinoid thiamethoxam and synthesized natural insecticides spinosad and abamectin are as effective as the contact insecticides used so far (Fang et al., 2002; Arthur et al., 2004; Athanassiou et al., 2008b; Kavallieratos et al., 2009; Andrić et al., 2011; Wakil et al., 2013).

Favourable toxicological and ecotoxicological traits of synthesized natural insecticides, especially of spinosad and abamectin, give these compounds a significant advantage and fair prospects for practical use. Spinosad is a combination of spinosyn A and spinosyn D, secondary metabolites of the soil actinomycete *Saccharopolyspora spinosa* (Mertz and Yao) (Thompson et al., 2000; Copping and Duke, 2007), and it has a significan role in organic food growing (Hertlein et al., 2011). It has been registered in the US, Australia and some African countries for use in storage facilities at a dose of 1 mg/kg (Subramanyam, 2006). Abamectin is a mixture of avermectin B_{1a} and B_{1b}, fermentation products of the actynomicete *Streptomyces avermitilis* (Kim and Godfellow) and it is used to protect a large number of crops from insect and mite pests (Copping and Duke, 2007; Krämer and Schirmer, 2007). However, it has not yet been registered for application in storages.

Some earlier studies have indicated that storage insects have different levels of susceptibility to spinosad and abamectin and that species belonging to the genus *Tribolium* are the least susceptible (Subramanyam et al., 1999; Kavallieratos et al., 2009; Vayias et al., 2009). Resistance to contact insecticides, as well as the geographic origin of populations of storage insects, have significant effects on their susceptibility to spinosad and abamectin (Fangeng et al., 2004; Nayak et al., 2005; Hussain et al., 2005; Athanassiou et al., 2008a).

Red flour beetle *Tribolium castaneum* Herbst, a secondary pest of stored plant products, is a species found both globally and in Serbia (Rees, 2004; Almaši, 2008). In this country, contact insecticides that have been used to control this species include malathion, dichlorvos, pirimiphos-methyl and deltamethrin (Kljajić, 2008; Janjić and Elezović, 2010). According to the latested research data (Andrić et al., 2010), some populations of *T. castaneum* in Serbia have developed resistance to malathion. Hence, our goal was to assess under laboratory conditions: (1) the efficacy of spinosad and abamectin as new insecticides for controlling adults of a laboratory population of *T. castaneum* with normal susceptibility to insecticides, and two populations (Nikinci and Jakovo) that are resistant to malathion, and (2) to detect potential differences in susceptibility to spinosad and abamectin between the test populations.

MATERIAL AND METHODS

Populations tested and insecticides used

A laboratory population of *T. castaneum* with normal susceptibility to insecticides and two populations resistant to malathion, Nikinci (warehouse) and Jakovo (silo) with respective resistance factors of LD_{50} 17.6 and 26.0 (Andrić et al., 2010), were used in the tests. All populations were reared in an insectarium according to methods described by Harein and Soderstrom (1966) and Davis and Bry (1985), i.e. in 2.5 L glass jars, on wheat flour containing 5% dry yeast, at a temperature of 25±1°C and relative air humidity of 60±5%. Adults aged 2-4 weeks, with undetermined sex ratio were used in the test.

The following commercial products were used in the experiment: Spinosad 240 SC (NAF-315) containing 22.8% spinosad (Dow AgroSciences, USA) and Abastate, EC formulation containing 18 g/L abamectin (Galenika-Fitofarmacija, Serbia).

Bioassays

Investigation was conducted in the laboratory (25±1°C and 60±5% r.h.) following a modified method described by Collins (1990) and a method for insecticide efficacy evaluation for storage pest control (OEPP/EPPO, 2004). Untreated soft wheat, variety Evropa, with 11.6±0.2% grain moisture content read on a Dickey-john moisture meter device (Dickey-john mini GAC Dickey-john Co.,USA) was used in the experiment.

The insecticides were applied to all tested populations at the following doses: 0.25, 0.5, 1.0, 2.5 and 5.0 mg a.i./kg by pipetting 5 mL of insecticide solution onto 500 g of wheat grains previously poured into each 1000 ml glass jar. After hand shaking for 30 s, each jar was placed on a mechanical roller for 10 minutes, each sample (500 g) of treated wheat was then divided into three equal subsamples (166 g) for each dose and each test population, and placed into 720 ml glass jars. Control wheat was treated the same way with water. The following day, 50 *T. castaneum* adults from each tested population were released into each jar, which was then covered with cotton cloth and fixed with rubber band. Mortality of individuals from the test populations was determined after 7, 14 and 21 days from the beginning of their exposure to treated wheat grain.

Data analysis

The acquired mortality data were adjusted for mortality in the control using Abbott's formula (1925). The mortality data were analyzed using the repeated measures ANOVA. Exposure intervals represented the repeated factor, while insect mortality was the response variable and populations, insecticides and application doses were the main effects. The means were separated by Fisher's LSD test at P<0.05. Before analysis, the percentage of mortality was transformed using *arcsine* (Sokal and Rohlf, 1995). However, untransformed means and standard errors are shown in the tables.

RESULTS

Mortality levels of *T. castaneum* were significantly affected during the exposure periods ($F_{2,120}$ = 1570,1; P < 0.0001). All main effects and the associated interactions for mortality levels of *T. castaneum* between and within exposure intervals were significant at *P* = 0.05 (Table 1).

Table 1. Repeated measures ANOVA parameters for main and associated interactions for mortality of *T. castaneum*

Main effects	df	F	P
Populations	2	3620.4	<0.0001
Insecticides	1	353.2	<0.0001
Doses	4	630.7	<0.0001
Populations x insecticides	2	19.1	<0.0001
Populations x doses	8	7.3	<0.0001
Insecticides x doses	4	32.7	<0.0001
All between	8	3.0	0.0067
Error	60		
Exposure	2	1570.1	<0.0001
Exposure x populations	4	18.1	<0.0001
Exposure x insecticides	2	200.0	<0.0001
Exposure x doses	8	210.7	<0.0001
Exposure x populations x insecticides	4	14.2	<0.0001
Exposure x populations x doses	16	3.4	<0.0001
Exposure x insecticides x doses	8	22.9	<0.0001
All between	16	2.7	0.0012
Error	120		

After 7 days of exposure, spinosad efficacy in all tested populations (Table 2) was low. The highest mortality (13.3%) was registered in population Nikinci on wheat treated with 5 mg a.i./kg. After the same exposure period, abamectin (Table 3) reached its highest lethal effect at doses of 2.5 and 5 mg/kg in the laboratory population (20% and 26%, respectively) and in the population from Jakovo (20% and 29%, respectively), while mortality was significantly lower statistically in population Nikinci (13% and 17%, respectively).

Table 2. Spinosad efficacy against *T. castaneum* adults from laboratory, Nikinci and Jakovo populations in treated wheat grain

Population	Dose (mg/kg)	Mortality (%±SE) after exposure		
		7 days	14 days	21 days
Laboratory	5.0	7.3±2.5 b	74.7±4.0 a	97.3±1.5 a
	2.5	2.0±0.0 cd	31.3±1.5 c	64.0±6.2 c
	1.0	0.7±0.6 d	2.0±0.0 ef	6.7±1.1 ef
	0.5	0.0±0.0 d	0.0±0.0 f	2.0±1.0 f
	0.25	0.0±0.0 d	0.0±0.0 f	0.0±0.0 f
Nikinci	5.0	13.3±2.5 a	46.7±3.5 b	86.7±4.0 b
	2.5	3.3±2.1 bcd	15.3±2.9 d	55.3±5.7 c
	1.0	1.3±0.6 cd	8.0±3.0 e	13.3±3.1 e
	0.5	0.0±0.0 d	0.0±0.0 f	2.0±0.0 f
	0.25	0.0±0.0 d	0.0±0.0 f	0.0±0.0 f
Jakovo	5.0	5.3±1.1 bc	71.3±2.3 a	88.0±3.5 ab
	2.5	4.0±2.0 bcd	26.7±3.1 c	44.7±2.1 c
	1.0	2.7±1.5 cd	4.7±1.5 ef	13.3±1.1 e
	0.5	0.0±0.0 d	0.0±0.0 f	2.7±1.1 f
	0.25	0.0±0.0 d	0.0±0.0 f	1.3±0.6 f

Means within columns followed by the same letter are not significantly different (Fisher's LSD test, p>0.05)

Table 3. Abamectin efficacy against *T. castaneum* adults from laboratory, Nikinci and Jakovo populations in treated wheat grain

Population	Dose (mg/kg)	Mortality (%±SE) after exposure		
		7 days	14 days	21 days
Laboratory	5.0	26.0±2.0 a	58.0±2.6 a	100 a
	2.5	20.1±2.1 b	54.0±3.0 a	94.7±3.8 a
	1.0	3.3±1.1 de	28.0±1.7 c	82.7±1.5 cd
	0.5	2.0±0.0 e	10.7±2.1 ef	40.1±2.3 f
	0.25	0.0±0.0 e	8.0±1.7 fg	18.0±2.6 g
Nikinci	5.0	17.3±2.3 b	44.7±5.0 b	86.0±3.0 bc
	2.5	12.7±0.6 c	25.3±1.5 cd	74.7±2.9 d
	1.0	3.3±1.1 de	18.0±3.6 de	53.3±2.5 e
	0.5	2.0±0.0 e	11.3±2.5 ef	34.0±3.0 f
	0.25	0.0±0.0 e	0.0±0.0 g	4.0±1.7 h
Jakovo	5.0	28.7±2.5 a	58.0±1.0 a	100 a
	2.5	20.0±2.0 b	54.0±4.0 a	94.0±3.0 ab
	1.0	7.3±1.1 d	25.3±1.5 cd	82.7±3.2 cd
	0.5	2.0±1.0 e	8.0±1.0 fg	40.0±1.7 f
	0.25	0.0±0.0 e	2.0±0.6 g	17.3±0.6 g

Means within columns followed by the same letter are not significantly different (Fisher's LSD test, p>0.05)

After 14 days of exposure, spinosad applied at 5 mg/kg (Table 2) caused the highest mortality in the laboratory and Jakovo populations (75% and 71%, respectively), while the same application dose caused a significantly lower mortality (47%) among adults from Nikinci. Also, spinosad applied at 2.5 mg/kg resulted in a significantly lower mortality of only 15% in the population from Nikinci, compared to mortality observed in the laboratory population (31%), and population originating from Jakovo (27%). After 14 days, abamectin applied at 2.5 and 5 mg/kg (Table 3) achieved the highest mortality in the laboratory and Jakovo populations (54% and 58%, respectively), while the same doses caused a significantly lower mortality (25% and 45%, respectively) in the population from Nikinci.

After 21 days, spinosad applied at 5 mg/kg reached its highest efficacy of 97% in the laboratory population, followed by 88% in the population originating from Jakovo, and lowest efficacy of 87% in population Nikinci (Table 2). In that time interval, the lower spinosad doses (0.25-2.5 mg/kg) caused no statistically significant differences in mortality among the tested populations. Abamectin (Table 3) applied at the doses of 2.5 and 5 mg/kg caused high mortality (94-100%) of adults in the laboratory and Jakovo populations after 21 days of exposure, while their mortality in population Nikinci was significantly lower (75% and 86%, respectively).

DISCUSSION

Data from this study show that the exposure period has a significant effect on spinosad and abamectin efficacy against *T. castaneum* adults and that their highest efficacy was achieved after 21 days of exposure, which is consistent with reports by Kavallieratos et al. (2009) and Vayias et al. (2009). In those studies, *Tribolium confusum* Jacquelin du Val adults had been found significantly less susceptible to spinosad and abamectin than *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.). Comparing data from the present study with our earlier research (Andrić et al., 2011), we also found that *T. castaneum* populations were significantly less susceptible to spinosad and abamectin than *S. oryzae* populations from Serbia, and that abamectin was significantly more effective than spinosad. Kavallieratos et al. (2009) had found that abamectin applied to wheat at the dose of 1 mg/kg caused high effectiveness (95%) against *T. confusum* adults after 21 days of exposure., In our study, however, abamectin was highly effective (95%) against *T. castaneum* adults at the dose of 2.5 mg/kg, but only against the laboratory and Jakovo populations.

Some earlier studies (Subramanyam et al., 1999; Fang et al., 2002; Nayak et al., 2005) have shown that *T. castaneum* is the least susceptible stored-product pest to spinosad and that the recommended dose of 1 mg/kg cannot control adults of this species. After a 14-day exposure period, Subramanyam et al. (1999) recorded a mortality of 52% among *T. castaneum* adults on wheat treated with spinosad at the dose of 6 mg/kg, and Fang et al. (2002) reported that a full control of *T. castaneum* adults required spinosad doses exceeding 4 mg/kg, while Huang and Subramanyam (2007) found a 98% efficacy of spinosad at 2 mg/kg after 12 days of contact of *T. castaneum* adults. In those studies, spinosad applied to wheat at the dose of 5 mg/kg reached 97% efficacy only against adults of laboratory populations of *T. castaneum*. Fangeng et al. (2004) found that, considering LD_{50}, two collected field populations of *Plodia interpunctella* (Hubner) from northeastern Kansas were both 1.7-fold less susceptible to spinosad, while two populations of *T. castaneum* were 4.8- and 7.6-fold less susceptible than a laboratory population. This indicates that geographic origin of populations of stored-product insect pests may significantly affect insecticide efficacy. Athanassiou et al. (2008a) found that larvae and adults of *T. confusum* from Greece and Italy were less susceptible to spinosad than populations from Germany and Denmark.

Resistance of some stored-product pests to one insecticide may significantly affect the efficacy of other insecticides. For example, Nayak et al. (2005) reported a 2.4-fold lower efficacy of spinosad (1 mg/kg) against a population of *S. oryzae* resistant to malathion than against laboratory population. In our own research, spinosad applied at the doses of 2.5 and 5 mg/kg was 2.0- and 1.6-fold less effective against the Nikinci population of *T. castaneum* after 14 days than it was against the laboratory population, while no significant difference was detected between the laboratory and Jakovo populations. The difference in abamectin efficacy between the laboratory population of *T. castaneum* and Nikinci population is statistically significant, while no significant difference was detected between the laboratory and Jakovo populations. The present data are not consistent with data from a study presented by Hussain et al. (2005), in which *T. castaneum* larve from a population resistant to malathion were significantly more susceptible than larvae from a population with unchanged susceptibility to malathion. The differences in susceptibility to spinosad and abamectin between the laboratory population of *T. castaneum* and Nikinici population, and especially the difference between the Nikinici and Jakovo populations (both resistant to malathion) are probably the result of genetic mutation and a considerable genetic heterogeneity of the population originating in Nikinci.

Besides these findings, our data also indicate a need to include insects from resistent populations in tests of new insecticides in order to get a full insight into potentials of each compound before including it in a subsequent pest management programs.

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Efikasnost spinosada i abamektina za različite populacije kestenjastog brašnara (*Tribolium castaneum* Herbst) u tretiranoj pšenici u zrnu

REZIME

U laboratorijskim uslovima (25±1ºC i 60±5% r.v.v.) je ispitivana efikasnost spinosada i abamektina za adulte kestenjastog brašnara iz laboratorijske populacije, normalno osetljive na kontaktne insekticide, i populacija Nikinci i Jakovo, rezistentne na malation. Za sve testirane populacije insekticidi su naneti na po 500 g netretirane pšenice u zrnu u istim dozama: 0,25; 0,5; 1,0; 2,5 i 5,0 mg a.m./kg. Posle nanošenja insekticida tretirana pšenica je deljena na tri jednaka dela da bi sutradan u svaku ispitivanu količinu, posebno, bilo ubacivano po 50 adulta kestenjastog brašnara svih testiranih populacija. Smrtnost je utvrđivana posle 7, 14 i 21 dana izlaganja u tretiranoj pšenici u zrnu.

Generalno, sa povećanjem doza i intervala izlaganja povećava se efikasnost oba insekticida, s tim da je abamektin značajno efikasniji od spinosada za sve tri testirane populacije. Posle 7 dana izlaganja ni u jednoj ispitivanoj varijanti nije utvrđena smrtnost veća od 30%, dok je posle 14 dana najveća smrtnost utvrđena kod laboratorijske populacije (75%), u pšenici tretiranoj najvišom dozom (5 mg/kg) spinosada, dok je tretman istom dozom abamektina najveću smrtnost (58%) prouzrokovao kod laboratorijske i populacije Jakovo. Posle 21 dana spinosad je najveću smrtnost ostvario primenom doze od 5 mg/kg kod laboratorijske populacije (97%), a zatim kod populacija Jakovo (88%) i Nikinci (87%). Abamektin je u dozama 2,5 i 5 mg/kg prouzrokovao visoku smrtnost (94-100%) adulta iz laboratorijske i populacije Jakovo i statistički značajno nižu smrtnost adulta iz Nikinaca (75 i 86%).

Utvrđene statistički značajne razlike u efikasnosti spinosada i posebno abamektina, između testiranih populacija, gde su najznačajnije razlike utvrđene između laboratorijske populacije i populacije Nikinci, jasno pokazuju da rezistentnost adulta kestenjastog brašnara na malation značajno utiče na efikasnost spinosada i, posebno, abamektina.

Ključne reči: Efikasnost; abamektin; spinosad; populacije; *T. castaneum*