Susceptibility of red flour beetle *Tribolium castaneum* (Herbst) populations from Serbia to contact insecticides

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Abstract

Contact insecticides remain the principal option for controlling stored-product insects. Unsatisfactory results of insecticide applications are caused by several factors, one of the most important being resistance of stored-product insects. The objective of this study was to examine the susceptibility in several populations of red flour beetle *Tribolium castaneum* (Herbst) from Serbia to different contact insecticides. Toxicity of the insecticides dichlorvos, malathion, chlorpyrifos-methyl, pirimiphos-methyl, deltamethrin and bifenthrin to adults of a laboratory population of *T. castaneum* was investigated in the laboratory by topical application. At the LD_{50}, deltamethrin was the most toxic and malathion the least toxic of the insecticides. Discriminating dose data for the laboratory population were used to test the susceptibility of 10 other populations originating from different storage facilities (silos, warehouses and flour mills) in Serbia. The discriminating dose of malathion caused mortality of up to 85% in seven populations, indicating malathion resistance in those populations. For two populations of *T. castaneum* from Nikinci and Jakovo LD values, *ld-p* lines and levels of susceptibility/resistance (RRs) were determined. The most toxic insecticide for adults from Nikinci and Jakovo was deltamethrin, while malathion was least toxic. The resistance ratios (RRs) for malathion at the LD_{50} were 17.6 for beetles from Nikinci, and 26.0 for beetles from Jakovo.

Keywords: *Tribolium castaneum* adults; Different populations; Insecticide toxicity; Susceptibility resistance

1. Introduction

Red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a cosmopolitan species and one of the most widespread insects in storage facilities worldwide, especially in mills, and it develops most rapidly under favourable conditions (Rees, 2004). In Serbia, *T. castaneum* is common in grain storages, mills and flour depots, as well as in foodstuff production plants (Almaši, 2008). Application of contact insecticides and fumigants continues to be the most significant tools for protection of stored products from insect pests. Contact insecticides ensure long-term protection and prevent infestation of treated products. Several formulations based on malathion, pirimiphos-methyl and synergized or nonsynergized deltamethrin have been registered in Serbia for treatment of grains, and dichlorvos for treatment of vacant storage areas (Kljajić, 2008).

The efficacy of contact insecticides in protecting stored products is affected by several factors, primarily by altered susceptibility or resistance of stored-product insects (Subramanyam and Hagstrum, 1996; Kljajić and Perić, 2005). According to data collected globally by the FAO organization (Champ and Dyte, 1976), susceptibility to malathion had changed in 87% of the examined populations of *T. castaneum*. In the USA, many populations collected from peanut, barley and wheat storages were resistant to malathion (Halliday et al., 1988; Subramanyam et al., 1989; Zettler and Cuperus, 1990). Populations of *T. castaneum* from mills were resistant to malathion, and also dichlorvos and chlorpyrifos-methyl (Zettler, 1991; Zettler and Arthur, 1997). For the purpose of monitoring *T. castaneum* resistance to insecticides in Serbian storage facilities, we determined the toxicity parameters (LD levels and *ld-p* lines) for a laboratory population of *T. castaneum* by topical application and determined levels of susceptibility/resistance (resistance ratios, RRs) in populations collected from different silos, warehouses and flour mills.

2. Materials and methods

2.1. Populations tested and contact insecticides used

A laboratory population of *T. castaneum* was used in the experiment, as well as populations collected in 2008 and 2009 from various storage facilities in Serbia: (1) Bačka Topola (silo and flour mill) and Čurug (silo) in the north of Serbian province of Vojvodina; Novo Miloševo (silo) and Kikinda (flour mill) in the northeast; and Adaševci (silo), Nikinci (warehouse) and Irig (silo) in the southwest part, and (2) Belgrade Port (silo) and Jakovo (silo) of Belgrade area.

All populations were reared using methods described by Harein and Soderstrom (1966) and Davis and Bry (1985). Two-to-four-week-old unsexed insects were used in all tests and F_3 generation of beetles for the susceptibility tests of collected *T. castaneum* populations. Technical concentrates of the following insecticide active ingredients were used in bioassay: dichlorvos 98%, malathion 96%, chlorpyrifos-methyl 97%, pirimiphos-methyl (product Actellic 50 EC containing 50% a.i.), deltamethrin 98% and bifenthrin 94.7%.

2.2. Bioassay

Insecticide toxicity to *T. castaneum* adults and susceptibility/resistance of the collected populations were tested by topical application in the laboratory at 24±1ºC and 60±5% r.h. (Halliday et al., 1988). To immobilize the insects, they were anesthetized with CO_2 for about 30 seconds before 0.5 µL of each insecticide, disolved in acetone (6-8 concentrations), was applied to the thorax of each insect by Burkard microapplicator (needle No. 18 in 1.0 mL syringe). Control insects were treated with aceton alone. After treatment of 25 insects in four replications, the beetles were transferred to clean Petri dishes after 4-6 h, each was filled with approximately 1 g of wheat flour. Lethal effects were determined after 7 d of microapplication. Based on discriminating doses causing 100% mortality in the laboratory population and reactions of each collected population to them, the populations from Nikinci and Jakovo were chosen for a full-range testing of insecticide toxicity or susceptibility.

2.3. Data analysis

Mortality data for treated insects were corrected for mortality in the control using Abbott's (1925) formula. Data were processed by probit analysis according to a method described by Finney (1971) and using a computer software developed by Raymond (1985). Statistical significance of differences between toxicity indicators for the insecticides investigated was assessed based on the overlapping/non-overlapping of intervals of confidence.

3. Results

Data on the toxicity of contact insecticides to adults of the laboratory population of *T. castaneum* (Table 1) show that deltamethrin was the most toxic insecticide at the LD_{50} (0.0069 µg insect $^{-1}$), i.e., 40.6 times more toxic than the lowest values recorded for malathion (0.28 µg insect $^{-1}$). At the LD_{95}, however, pirimiphos-methyl (0.0018 µg insect $^{-1}$) demonstrated the highest toxicity, being as much as 3,300 times more toxic than malathion at the lowest level (5.97 µg insect $^{-1}$) of the tested insecticides.

Table 1 Insecticide toxicity to *T. castaneum* adults from a laboratory population assayed by topical application after 7 d of exposure.

Insecticide	LD_{50} (µg insect $^{-1}$) Fiducial Limits (0.05)	LD_{95} (µg insect $^{-1}$) Fiducial Limits (0.05)	Slope *ld-p* line (±SE)	DD* (µg insect $^{-1}$)
Dichlorvos	0.040 (0.038-0.043)	0.087 (0.079-0.097)	4.98±0.34	0.12
Malathion	0.28 (0.21-0.36)	5.97 (4.08-9.66)	1.24±0.08	10.0
Chlorpyrifos-methil	0.011 (0.0104-0.0115)	0.019 (0.018-0.021)	6.52±0.42	0.030
Pirimiphos-methil	0.0091 (0.0087-0.0095)	0.0018 (0.017-0.019)	5.68±0.27	0.025
Deltamethrin	0.0069 (0.0062-0.0075)	0.024 (0.021-0.027)	3.05±0.15	0.050
Bifenthrin	0.049 (0.041-0.056)	0.27 (0.22-0.35)	2.19±0.16	0.50

*Discriminating dose

Discriminating doses of the insecticides dichlorvos, pirimiphos-methyl, chlorpyrifos-methyl, deltamethrin and bifenthrin (Table 2) caused high mortality in all collected populations of *T. castaneum*, ranging from 92-100%. The discriminating dose of malathion caused 100% mortality only in a population from Bačka Topola (flour mill), and high mortality in another Bačka Topola population (silo) and one from Belgrade Port (silo), 95 and 99%, respectively. Mortality in all other populations was below 85%, and the lowest in the Jakovo population (silo), up to 64%.

Table 2 Effects of discriminating doses of insecticides to *T. castaneum* adults from different populations assayed by topical application after 7 d of exposure.

	Mortality (%±SE)					
Population	**DDVP**	**PM**	**CPM**	**MAL**	**DEL**	**BIF**
Bačka Topola-FM	99±0.5	99±0.5	100	100	94±1.3	96±0.8
Bačka Topola-S	97±1.0	93±1.0	92±0.8	95±0.5	96±0.8	100
Belgrade Port-S	99±0.5	93±1.5	100	99±0.5	96±1.2	100
Čurug-S	99±0.5	92±2.2	99±0.5	80±0.8	98±0.6	100
Nikinci-W	98±0.6	94±1.3	100	70±2.5	99±0.5	100
Jakovo-S	100	99±0.5	100	64±2.9	100	100
Adaševci-S	99±0.5	97±0.5	100	79±2.2	100	100
Novo Miloševo-S	99±0.5	99±0.5	98±0.6	80±0.8	98±0.6	100
Irig-S	93±1.0	94±0.6	96±1.2	74±2.4	98±0.6	97±1.0
Kikinda-FM	100	99±0.5	98±1.0	84±2.2	96±1.4	100

S-silo; W-warehouse; FM-flour mill

Tables 3 and 4 show the results of insecticide toxicity (LD levels and *ld-p* lines) and resistance ratios (RRs) for beetles from Nikinci and Jakovo. For the *T. castaneum* from Nikinci, deltamethrin was the most toxic insecticide (0.0066 µg insect $^{-1}$) at the LD_{50}, and chlorpyrifos-methyl (0.016 µg insect $^{-1}$) was most toxic at the LD_{95}, which were 748.5 and 4097.5 times more toxic, respectively, than the lowest value for malathion (LD_{50} 4.94 µg insect $^{-1}$and LD_{95} 65.56 µg insect $^{-1}$).

For *T. castaneum* adults from Jakovo deltametrin (0.0054 µg insect $^{-1}$) was the most toxic insecticide at the LD_{50}, while pirimiphos-methyl (0.0163 µg insect $^{-1}$) was most toxic at the LD_{95}, and they were 1348.1 and 2238.0 times more toxic, respectively, than the lowest toxicity for malathion (LD_{50} 7.28 µg insect^{-1} and LD_{95} 36.48 µg insect $^{-1}$). The RRs show that both populations were resistant to malathion as its RRs were 17.6 and 26.0 at the LD_{50}, and 11.0 and 6.1 at the LD_{95} for beetles from Nikinci and Jakovo, respectively.

Table 3 Insecticide toxicity to *T. castaneum* adults from Nikinci population assayed by topical application after 7 d of exposure and resistance ratios found (RR = LD tested/LD laboratory population).

Insecticide	**LD_{50} (µg insect $^{-1}$) Fiducial Limits (0.05)**	**RR for LD_{50} level**	**LD_{95} (µg insect $^{-1}$) Fiducial Limits (0.05)**	**RR for LD_{95} level**	**Slope *ld-p* line (±SE)**
Dichlorvos	0.042 (0.039-0.044)	1.0	0.087 (0.080-0.096)	1.0	5.15±0.33
Malathion	4.94 (4.23-5.72)	17.6	65.56 (49.25-93.58)	11.0	1.46±0.01
Chlorpyrifos-methil	0.0098 (0.0093-0.010)	0.9	0.016 (0.015-0.018)	0.8	7.21±0.60
Pirimiphos-methil	0.011 (0.010-0.011)	1.2	0.025 (0.022-0.029)	1.4	4.48±0.34
Deltamethrin	0.0066 (0.0058-0.0073)	0.9	0.029 (0.025-0.035)	1.2	2.53±0.17
Bifenthrin	0.054 (0.049-0.060)	1.1	0.14 (0.11-0.17)	0.5	4.07±0.37

Table 4 Insecticide toxicity to red flour beetle adults from Jakovo population assayed by topical application after 7 d of exposure and resistance ratios found (RR = LD tested/LD laboratory population).

Insecticide	LD_{50} (µg insect $^{-1}$) Fiducial Limits (0.05)	RR for LD_{50} level	LD_{95} (µg insect $^{-1}$) Fiducial Limits (0.05)	RR for LD_{95} level	Slope *ld-p* line (±SE)
Dichlorvos	0.035 (0.033-0.037)	0.8	0.079 (0.071-0.090)	0.9	4.65±0.34
Malathion	7.28 (6.54-8.03)	26.0	36.48 (30.85-44.85)	6.1	2.35±0.14
Chlorpyrifos-methil	0.012 (0.0113-0.0127)	1.1	0.024 (0.022-0.027)	1.3	5.55±0.40
Pirimiphos-methil	0.0090 (0.0085-0.0094)	0.9	0.0163 (0.0154-0.0176)	0.9	6.31±0.38
Deltamethrin	0.0054 (0.0047-0.0060)	0.7	0.024 (0.020-0.029)	1.0	2.55±0.19
Bifenthrin	0.040 (0.034-0.045)	0.8	0.17 (0.14-0.21)	0.6	2.65±0.23

4. Discussion

As in many other studies worldwide, our experiments confirmed resistance to malathion. This is based on the effects of discriminating doses of contact insecticides on the ten tested populations of *T. castaneum,* and toxicity parameters and RRs for populations from Nikinci and Jakovo. However, compared to data on species resistance to insecticides summarized by Subramanyam and Hagstrum (1996) and Kljajić and Perić (2005), the level of resistance to malathion was low in the two examined populations in our experiment (RRs 17.6 and 26.0 at the LD_{50} for beetles from Nikinci and Jakovo, respectively).

Subramanyam et al. (1989) had examined the susceptibility of *T. castaneum* and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) populations collected from barley warehouses using discriminating doses of malathion, pirimiphos-methyl and chlorpyrifos-methyl applied to filter paper. All tested populations of *T. castaneum* displayed resistance to malathion, but no cross resistance to either pirimiphos-methyl or chlorpyrifos-methyl.

In a study by Halliday et al. (1988) using topical application of discriminating doses of malathion, dichlorvos, pirimiphos-methyl, chlorpyrifos-methyl and synergized pyrethrins, several populations of *T. castaneum* from stored peanuts were resistant to malathion alone, mortality in 12 of the 15 tested population below 10%. Zettler (1991) used the same method to test susceptibility of *T. castaneum* and *Tribolium confusum* Jacquelin du Val populations collected from mills in the USA to malathion, dichlorvos, chlorpyrifos-methyl, synergized pyrethrins and resmethrin. Of the 28 tested populations of *T. castaneum*, 93% were resistant to malathion, 64% to dichlorvos, 36% to chlorpyrifos-methyl, while none were resistant to synergized pyrethrins or resmethrin.

More recently, Zetller and Arthur (1997) tested the susceptibility of 14 populations of *T. castaneum* and 10 populations of *T. confusum* from various mills to malathion and dichlorvos and found all *T. castaneum* populations to be resistant to dichlorvos and even more to malathion (top RR=29,081). They found a positive correlation between insect survival after exposure to discriminating doses of insecticides and lethal dose values.

In conclusion, our results confirmed the importance monitoring the susceptibility/resistance of *T. castaneum* and other stored-product insects to insecticides as an important element of pest management programs.

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