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Toxicity and repellency of dimethoate, pirimiphos-methyl and deltamethrin against *Tribolium castaneum* (Herbst) using different exposure methods

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A B S T R A C T

Tribolium castaneum, the red flour beetle, is one of the major pests of cereal grains and their products. Infestation of raw food products by *T. castaneum* can be tackled by use of insecticides which offer a rapid and effective control method for different insect pests. In the present study, toxicity and repellency of three widely used insecticides was assessed on adult red flour beetles to investigate how a flour substrate affects the results of the standard laboratory toxicity and repellency test methods. The red flour beetles were exposed to the organophosphates dimethoate and pirimiphos-methyl and the pyrethroid deltamethrin, following the original and two modified residual film methods (toxicity assessment) and by the area preference method to assess repellency. Since the conditions of exposure (glass and filter paper surfaces as exposure substrates) in the original methods are quite different compared to the exposure carried out in stored-product protection, methods were modified to include flour as an exposure substrate. The results showed that according to the LC_{50} values, toxicity of the investigated insecticides could be arranged in the following order: pirimiphos-methyl > dimethoate > deltamethrin. Also, both mortality and repellency were dependent on the exposure methods, i.e. presence and treatment of flour substrate. Mortality was significantly reduced in comparison to the original method. Repellency was recorded when beetles were exposed to deltamethrin using flour as a substrate, whereas using the filter paper surface (original method) repellency was not obtained. The results of the present study clearly indicate that there is an influence of substrate on the susceptibility of *T. castaneum* to insecticides and that the efficacy of different insecticides is affected by exposure substrate.

1. Introduction

Tribolium castaneum (Herbst), the red flour beetle, is a polyphagous, cosmopolitan pest species belonging to the family Tenebrionidae (darkling beetles), order Coleoptera. *Tribolium castaneum* is one of the major pests of cereal grains and their products. This species causes heavy losses and damage to different types of food grains. Besides the physical damage, the scent glands of the adult beetles secrete carcinogenic quinones which can also cause allergenic diseases (Lu et al., 2010). It is a very prolific, cosmopolitan, long-lived species quite resistant to eradication. Infestation of food processing facilities and incoming food products can be controlled by appropriate application of insecticides which offer a rapid and effective control method for insect pests.

Since *T. castaneum* is easy to culture and handle in the laboratory, this species has been used widely as an animal model in population ecology and toxicology studies (Alam et al., 2009; Abu Osman et al., 2011; Lale et al., 2000). In the present study, the toxicity and repellency of three widely used insecticides, two organophosphates, dimethoate and pirimiphos-methyl, and the pyrethroid deltamethrin, were investigated on adult red flour beetles. Organophosphates are widely used insecticides which all share a common mechanism of cholinesterase inhibition. Pyrethroids are synthetic insecticides similar to natural pyrethrins, of which the main effect is disruption of an insect's nervous system leading to loss of co-ordination and death (Mujeeb and Shakoori, 2012). Several previous studies have investigated the influence of these insecticides on *T. castaneum* (Lemon, 1966; Desmarchelier, 1977; Daglish et al., 1992; Arthur, 1994; Daglish, 1998; Mujeeb and Shakoori, 2012). According to Lagisz et al. (2010), pirimiphos-methyl is effective against red flour beetles even at a low application rate, but it also has a rapid pick-up time and delayed toxicity.

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Singh and Prakash (2013) recorded that deltamethrin is effective against *T. castaneum* at low concentrations, but resistance is quick to occur.

The most common method used to investigate the effects of insecticides on *T. castaneum* is the residual film method (Busvine, 1971). In this method insecticides are dissolved in acetone, applied on the bottom of Petri dishes and beetles are exposed on a thin film of insecticide that remains on the bottom after acetone evaporation. Since beetles are exposed to insecticide applied on a glass surface, the conditions of such exposure are quite different compared to the exposure experienced by *T. castaneum* in stored-product protection. In storage insecticides are often applied on different types of grains. With a view to establish the possible differences in pesticide toxicities dependent on the exposure substrate, besides the original method, modified methods were also applied and the main difference compared to the original method was the presence of flour as a substrate.

In addition to causing mortality of target organisms, pesticides may have various other effects, including repellency. When planning effective control strategies, studies on such subsidiary effects of insecticides on stored product pest populations are of considerable importance. Biotic factors, including insecticide repellency and behavioral avoidance of insecticide treatments, could be of comparable importance (Watson and Barson, 1995). If an insect's behavior is modified to avoid an insecticide-treated surface, its chances of survival could be greatly increased, so the insecticide treatment becomes less effective and the control of the pest population becomes more difficult (Pinniger, 1975; Barson et al., 1992). So far, repellency against stored product beetle pests has mostly been examined using plant essential oils, while there is a smaller number of studies evaluating the repellency of synthetic insecticides. Only a few studies investigated the behavior modifying effects of insecticides against *T. castaneum* (e.g. Prickett and Ratcliffe, 1977; Wildey, 1984, 1987).

In the present study, sublethal concentrations of three insecticides were evaluated for their possible repellent effect on *T. castaneum*. The repellent activity was measured using the area preference method (McDonald et al., 1970) in which the repellent effects are assessed by using the filter paper divided into two parts. This method has recently been used for repellency assessment of different essential oils against *Sitophilus zeamais* Motshulsky and *Tribolium confusum* J. du Val (Caballero-Gallardo et al., 2011; Tapondjou et al., 2005). Similar to the case with the residual film method, the exposure of beetles on filter paper in the original method poorly resembles exposure in storages, so a modified method for repellency assessment was applied in which adult *T. castaneum* were exposed using flour as a substrate.

The aim of the present research was to investigate the differences between toxicity and repellency results between standard laboratory test methods and modified methods in which flour was used as a substrate. Red flour beetles were exposed to the insecticides dimethoate, pirimiphos-methyl and deltamethrin and besides the residual film method (toxicity assessment) and area preference method (repellency assessment), modified methods were applied and the influence of flour as an exposure substrate on pesticide efficacy was assessed.

2. Materials and methods

2.1. Rearing of the test insects

The red flour beetles used for the tests were obtained from cultures that had been maintained for the previous two years in our laboratory. Insect cultures were maintained in jars with food medium at 30 ± 1 °C and relative humidity (r.h.) 70–80%. A mixture of white rough wheat flour with powdered dry yeast in a ratio 10:1 was used as food medium.

2.2. Chemicals

The following pesticides were used in this experiment: (1) Chromgor 40 (Chromos Agro d.d., Zagreb, Croatia), a dimethoate preparation (400 g/L of active ingredient); (2) Actellic 50 EC (Syngenta Agro d.o.o., Zagreb) a pirimiphos-methyl preparation (500 g/L of active ingredient); (3) Rotor 1.25 EC (Chromos Agro d.d., Zagreb), a deltamethrin preparation (12.5 g/L of active ingredient).

2.3. Toxicity tests

A preliminary screening of different doses of each insecticide was performed to obtain the range for 0–100% mortality. Insecticides were prepared separately in acetone to get concentrations of 1, 5, 10, 17.5, 25, 37.5, 50, 75, 100 and 1000 μg/mL and 2 mL of each dilution (concentration) was applied to Petri dishes. If expressed in μg/cm^2, the applied concentrations approximately corresponded to 0.03, 0.15, 0.3, 0.525, 0.75, 1.125, 1.5, 2.25, 3 and 30 μg/cm^2. In the original residual film method (Busvine, 1971) the adult red flour beetles were exposed to insecticide film produced in this way on a Petri dish. The principal modification in the present tests was the addition of 0.5 g of white rough wheat flour. Two modified methods were applied: 1) the beetles were exposed to insecticide dose mixed into 0.5 g flour and allowed to dry (abbreviated here onwards as "insecticide mixed into flour") and 2) the beetles were exposed to insecticide film applied on Petri dish covered with 0.5 g of flour after air drying (abbreviated later as "insecticide film covered with flour"). All methods were conducted by applying 10 different doses of each insecticide separately. Ten adult red flour beetles were then released within each Petri dish. A control batch was also maintained with the same number of insects after applying and evaporating only acetone. The treated insects were left in a dark place at room temperature. Mortality was recorded after 24 and 48 h of treatment.

2.4. Repellency bioassay

The area preference method, first described by McDonald et al. (1970), was used for assessment of repellency. Insecticides were prepared separately in acetone to obtain 1 μg/mL of pirimiphos-methyl, 50 μg/mL of dimethoate and 10 μg/mL of deltamethrin, and a volume of 1 mL was uniformly applied to a half-filter paper disk. If expressed in μg/cm^2, these concentrations approximately corresponded to 0.03, 1.5 and 0.3 μg/cm^2. The concentrations were determined based on preliminary tests conducted with each insecticide. On the other half of the filter paper an equal volume of acetone was applied and used as control (untreated). The treated and control half disks were air-dried for 10 min to evaporate the solvent, re-attached with adhesive tape, and kept in glass Petri dishes. Ten adult red flour beetles were released at the center of each filter paper disk. Dishes were covered and placed in darkness at room temperature. The numbers of *T. castaneum* specimens present on treated and untreated areas of the filter paper halves were counted 24 h after exposure. Six replicates were used for each concentration of each insecticide.

In the modified repellency method, the solutions of insecticides were prepared in acetone and a volume of 10 mL was applied on 100 g of white flour placed in glass Petri dishes. In control (untreated), an equal volume of acetone was added to the flour. The flour was then manually stirred to distribute the pesticide solution uniformly inside the flour and left for 1 h to evaporate the solvent. After the solvent evaporated, the flour was placed in the plastic