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Mortality and GST Enzyme Response of Saw-toothed Grain Beetles, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) Exposed to Low Insecticide Concentrations

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ABSTRACT

The saw-toothed grain beetle, *Oryzaephilus surinamensis* is a cosmopolitan insect pest that attacks stored grains and dried fruits, causing damage to stored dates in the United Arab Emirates (UAE). Stored dates may contain insecticide residues as a result of insecticide field applications and it is of interest to know the effect of insecticide residues on *O. surinamensis*. The aim of this study was to evaluate mortality among *O. surinamensis* adults exposed to residual levels of the insecticides carbosulfan, chlorpyrifos, cypermethrin, imidacloprid, malathion and spinosad which are commonly used on date palm trees in the UAE. Also to evaluate the Glutathione S-Transferase (GST) enzyme activity in the *O. surinamensis* adults exposed to insecticides. Adults of *O. surinamensis* were subjected to petri dish insecticide bioassays. Low insecticide concentrations, simulating field residues, were tested and the range of 0.713-3.31 μg mL^{-1} caused 50% mortality. Chlorpyrifos was the most toxic insecticide while cypermethrin was the least toxic. In addition to effect on mortality, insecticide exposure caused induction in GST enzyme activity in *O. surinamensis* adults. Because elevated GST enzyme activity can play a role in the detoxification of insecticides and in the development of insecticide metabolic resistance in insects it is likely to have a similar role in *O. surinamensis* population.

Key words: *Oryzaephilus surinamensis,* GST, specific activity, insecticide

INTRODUCTION

The saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) is the most widely distributed stored grain pest in the world. This insect attacks stored dates in the United Arab Emirates (UAE) (Zaid *et al*., 2002) and significantly lowers their market value. Several insect pests such as the red palm weevil, lesser date moth and date root borer attack date palm trees in the UAE and farmers use a number of insecticides to control them. Residues of some of these insecticides have been detected in ripe dates at concentrations reaching up to 5 ppm (μg mL^{-1}) (Al-Dhaheri, 2011). Consequently, *O. surinamensis* and other stored date insects are exposed to these residues while feeding on stored dates. The exposure of insects to insecticides causes mortality and also can act as a selection pressure for resistance development in the treated insect population (Georghiou *et al*., 1987; Ahmad *et al*., 2007). Resistance to an insecticide can be defined as a genetic change in response to selection by toxicants that may impair control in the field (Yu, 2008). Several detoxifying enzymes confer insecticide metabolic resistance in insects.

Glutathione S-transferases (GSTs) are one the major detoxifying enzymes associated with insecticide metabolic resistance (Bull, 1981; Oppenoorth, 1985). In general, GSTs are involved in the detoxification of various xenobiotic chemicals (Motoyama and Dauterman, 1980) and act as catalysts for the conjugation of various electrophilic compounds with tripeptide glutathione (Chasseaud, 1979; Wilce and Parker, 1994). Resistant insects to all major classes of insecticides often exhibit elevated GST activity (Huang *et al*., 1998; Vontas *et al*., 2001). The objectives of this study were (1) to evaluate mortality among *O. surinamensis* adults exposed to residual levels of six insecticides commonly used on date palm trees in the UAE and (2) to evaluate the GST enzyme activity in the *O. surinamensis* adults exposed to insecticides.

MATERIALS AND METHODS

The study was conducted in 2010 at the Entomology laboratory of the UAE University and lasted for four months.

Insects: The *O. surinamensis* adults were collected from infested dates and were placed inside an incubator at 25°C in screw cap plastic jars (2500 mL) containing insecticide-free dates as food.

Chemicals: Insecticide reference standards (chlorpyrifos, imidacloprid, spinosad, carbosulfan, malathion and cypermethrin) (Fig. 1) all from Dr. Ehrenstorfer GmbH, (Augsburg, Germany) were purchased from a local supplier. Acetone was used for dissolving the insecticides.

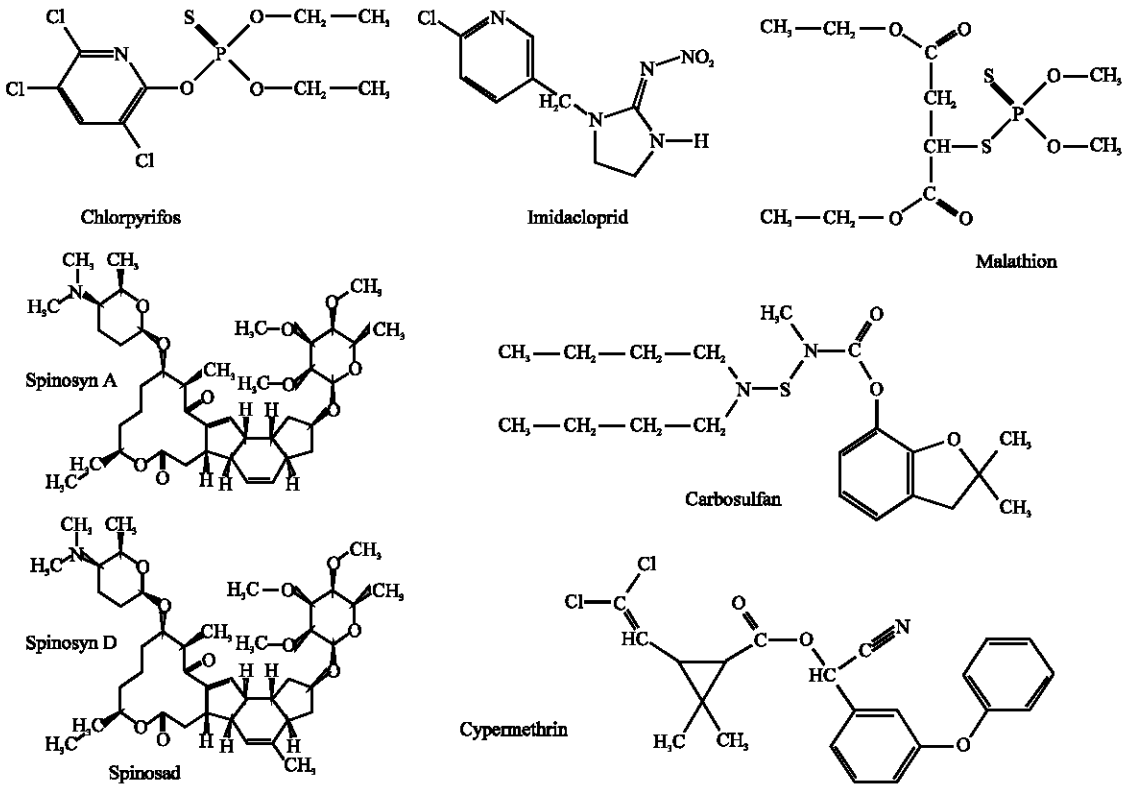

Fig. 1: Structures of the insecticides used in the petri dish residue bioassay on *O. surinamensis* adults, Spinosad is a mixture of spinosin A and D

Insecticide bioassay

LC_{50} tests: Mortality of *O. surinamensis* adults exposed to each one of the six insecticides was evaluated using a petri dish residue bioassay. For each bioassay, three replicates with at least seven concentrations of each insecticide were prepared. Each glass petri dish (Pyrex®, 100×15 mm) was treated with 1 mL of an insecticide dissolved in acetone. Treated dishes were left to air dry in a fume hood. Petri dishes treated only with acetone served as a control. After the dishes dried, a group of 15 unsexed *O. surinamensis* adults were transferred into each petri dish with a fine paintbrush and the dish was covered with its lid. Mortality was assessed after a 24 h waiting interval at 25°C with 12:12 (L:D) photoperiod. Adults of *O. surinamensis* were considered dead if they failed to walk after light tapping on the petri dish or being probed by the paintbrush. Mortality was corrected according to mortality of the controls using Abbott's formula (Abbott, 1925). The values of the LC_{50}, their 95% Confidence Intervals (CIs) and slopes of regression lines were estimated using PROBIT analysis (SPSS program; SPSS Inc., Chicago, IL). LC_{50} values of the tested insecticides were considered not significantly different if the 95% CIs overlapped.

Glutathione S-transferase enzyme activity bioassay

Exposure to low insecticide concentrations: A GST bioassay was conducted after determining the LC_{50} values for all tested insecticides. A concentration (0.5 μg mL^{-1}) which was lower than the least LC_{50} value among all of the tested insecticides was selected and used in this bioassay which was done before conducing the GST enzyme activity test. This concentration was chosen because it left an adequate number of live *O. surinamensis* adults for preparing an enzyme homogenate and also simulated exposing *O. surinamensis* adults to low post-harvest insecticide residue levels that might be encountered when feeding on stored date palm fruits. Adults of *O. surinamensis* were exposed to the selected concentration in a petri dish bioassay as described above.

Enzyme preparation: After exposure to insecticides the surviving insects were collected and homogenized in a potassium phosphate buffer (pH 6.5) using a ceramic mortar placed on ice. The homogenate was centrifuged at 13,200 rpm at 4°C for 10 min and the supernatant was decanted and used in the GST enzyme activity assays.

Glutathione S-transferase assay: The specific activity of GST of *O. surinamensis* adults was determined by the spectrophotometric method of Habig *et al.* (1974) using CDNB (1-chloro-2,4-dinitrobenzene) as a substrate. Each cuvette contained an insect homogenate (250 μL) mixed with potassium phosphate buffer (500 μL). Cuvettes were incubated at 25°C for 5 min. Ethanol-dissolved CDNB (10 μL; 0.2 M) and reduced glutathione (GSH) (150 μL; 10 mM) then were added. After 1 min, absorbance was measured at 340 nm using a spectrophotometer (Lambda 25, Perkin Elmer). A cuvette containing all reactants except substrate was used as a blank. The assay was done in triplicate. GST specific activity was calculated as ΔOD/min/mg protein (Tomarev *et al.*, 1991). Protein concentration of the insect homogenate was determined by the method of Bradford (1976) using Bovine Serum Albumin (BSA) as a standard. Data were analyzed using analysis of variance with insecticide treatments as the classification variable (SAS, 2001).

RESULTS

Insecticide bioassay: Table 1 shows the LC_{50} values of the tested insecticides. Chlorpyrifos was significantly the most toxic insecticide to *O. surinamensis* with an LC_{50} value of 0.713 μg mL^{-1},

Table 1: Mortality of *O. surinamensis* adults determined by petri dish bioassay

Insecticide	LC_{50} µg mL^{-1} (95% CI)[a]	Slope±SEM	χ^2 (df)	$p>\chi^{2b}$
Chlorpyrifos	0.713 (0.495-1.045)	2.072±0.335	2.969 (5)	0.71
Imidacloprid	3.122 (2.390-4.181)	4.819±1.253	6.271 (5)	0.28
Spinosad	2.562 (1.860-3.767)	2.919±0.620	1.037 (5)	0.96
Carbosulfan	1.637 (1.192-2.276)	2.910±0.543	3.245 (5)	0.662
Malathion	1.197 (0.939-1.587)	4.908±1.056	1.273 (5)	0.938
Cypermethrin	3.310 (2.259-6.148)	2.283±0.531	1.134 (5)	0.951[a]

The LC_{50} values are expressed in micrograms of active ingredient of insecticide per milliliter of acetone and their 95% confidence intervals (95% CI). [b]A value of $p>\chi^2$ larger than or equal to 0.05 indicates a significant fit between the observed and expected regression lines. LC_{50} values of the tested insecticides were considered not significantly different if the 95% CIs overlapped each other

Table 2: Mean (±SE) of GST specific activity (ΔOD/min/mg protein) in *O. surinamensis* adults treated with different insecticides at concentration of 0.5 µg mL^{-1}

Insecticide	GST specific activity
Cypermethrin	0.082±0.012[a]
Malathion	0.070±0.010[ba]
Carbosulfan	0.042±0.007[bc]
Chlorpyrifos	0.029±0.002[c]
Imidacloprid	0.028±0.002[c]
Spinosad	0.028±0.002[c]
Control	0.024±0.001[c]

Means with different letters are significantly different at $p<0.05$

followed by malathion and carbosulfan at 1.197 and 1.637 µg mL^{-1}, respectively. Spinosad was less toxic with an LC_{50} value of 2.562 µg mL^{-1}, whereas imidacloprid and cypermethrin were the least toxic with LC_{50} values of 3.122 and 3.310 µg mL^{-1}, respectively.

Glutathione S-transferase enzyme assay: Insecticide exposure caused significant differences in GST specific activity of *O. surinamensis* adults ($F = 4.85$, $df = 9, 20$, $p = 0.0016$) (Table 2). GST activity of cypermethrin-treated insects was not significantly higher than GST activity of malathion-treated insects ($t = -0.9004$, $p = 0.3786$). However, GST activity of cypermethrin-treated insects was significantly higher than in carbosulfan-treated insects ($t = -2.9416$, $p = 0.0081$).

The GST activity of *O. surinamensis* adults treated with spinosad, imidacloprid and chlorpyrifos did not significantly differ from the control [($t = 0.2766$, $p = 0.7841$), ($t = 0.2742$, $p = 0.7867$), ($t = 0.3397$, $p = 0.7376$), respectively]. Overall, the highest GST induction was caused by exposure to cypermethrin (activity was 3.4 fold of the control) followed by malathion (2.9 fold of the control). GST induction by malathion and cypermethrin was significantly higher than in the control insects [($t = 3.3032$, $p = 0.0035$) and ($t = 4.2037$, $p = 0.0004$), respectively].

DISCUSSION

Insecticide bioassay: All the values of $p>\chi^2$ were larger than 0.05 indicating a significant fit between the observed and expected regression lines of all the tested insecticides. The *O. surinamensis* population was homogenous in terms of its response to insecticide exposure for all the tested insecticides, indicated by slopes >2. A flat line or a low slope (<1), is indicative of a heterogeneous population, showing a large variance in response (Yu, 2008).

Pesticide residues can be found in fresh fruits as well as in their products (Cabras and Conte, 2001). The tested insecticides in the current study are commonly applied on date palm trees in the

UAE. The examined range of concentrations (0.05-5 μg mL^{-1}) simulated the residue levels that can be found on dates collected from insecticide treated date palm trees. Exposure to such concentration levels can cause mortality among *O. surinamensis,* when feeding on dates in storage. This was manifested in the current study by recording 50% mortality caused by a concentration range of 0.713 μg mL^{-1} for chlorpyrifos and 3.310 μg mL^{-1} for cypermethrin. Chlorpyrifos was significantly the most toxic insecticide based on its LC_{50} value (0.713 μg mL^{-1}) which indicates that the presence of this residue level in stored dates can cause a similar mortality in the *O. surinamensis* population. In accordance, chlorpyrifos-methyl and malathion (Lee and Lees, 2001) and spinosad (Fang *et al*., 2002) were reported causing mortality to *O. surinamensis*. Based on UAE weather conditions (temperature and humidity) and agricultural practices, harvested dates could contain insecticide residues reaching 5 μg mL^{-1} for some insecticides (Al-Dhaheri, 2011). Thus the presence of these insecticide residues can provide partial control against the insects including *O. surinamensis* that feed on stored dates. Although some insecticides such as chlorpyrifos were toxic to *O. surinamensis* at low concentrations and as mentioned can provide partial control, it is not recommended to apply insecticides to stored dates as a control measure because of human health risks. It was demonstrated that they show a potential dose-related chronic and acute toxicity against humans (Schilter and Huggett, 1998). If needed, such chemicals may be used to treat the walls and floor of the storeroom. From a pest management standpoint, the presence of insecticide residues at low concentrations, coupled with the extended exposure to these chemicals during the feeding on the dates in storage, may lead to development of insecticide resistance in *O. surinamensis* populations. Although the presence of insecticide residues can help in reducing the infestation of *O. surinamensis* in stored dates, it is better for date growers to use the recommended dose in the field to avoid human health risks and to delay the development of insecticide resistance.

Glutathione S-transferase (GST) enzyme assay: Glutathione S-transferase enzymes are useful biomarkers for metals and organic pollutants yielding oxidative stress (Yang *et al*., 2001) and have also been useful as an indicator of pesticide exposures (Taysse *et al*., 1998). They also have a significant role in insect resistance to Organophosphate (OP) insecticides (Motoyama and Dauterman, 1980). The results of the current study demonstrated that exposing *O. surinamensis* adults to insecticides at concentrations of 0.5 μg mL^{-1} caused a GST enzyme response in the form of activity induction. The maximum induction was caused by cypermethrin which is a pyrethroid insecticide. This insecticide was the least toxic to *O. surinamensis* based on its LC_{50}. The high GST activity in *O. surinamensis* adults exposed to cypermethrin likely explains its low toxicity. This is because GST enzymes play a role in xenobiotic metabolic detoxification. In addition, the high GST activity indicates that this enzyme group participates in the detoxification of cypermethrin and malathion to a greater extent than it does in the other tested insecticides. The induction of GST enzyme activity in this study was in agreement with the findings of previous studies. Yadwad and Kallapur (1988) evaluated the effect of fenitrothion on the castor semilooper *Achaea janata* (Lepidoptera: Noctuidae) and measured the GST activity. Both sublethal and lethal doses of fenitrothion produced significant induction of enzyme activity. Sivori *et al*. (1997) reported a significant increase of GST activity in *Triatoma infestans* (Heteroptera: Reduviidae) adults which were toxicated with sublethal dose of tetramethrin. Not all of the tested insecticides significantly induced GST enzyme activity in *O. surinamensis* and this is in part because they belong to different insecticide chemical groups (pyrethroids, organophosphates and nicotinoids). Moreover,

other detoxifying enzymes such as cytochrome P450 and esterases can also be involved in the detoxification process of insecticides. It is also likely that the 0.5 μg mL^{-1} concentration was not sufficient for some of the tested insecticides to cause induction in the GST enzyme activity.

CONCLUSION

In conclusion, exposure to date palm fruits containing insecticide residues at low concentrations can cause up to 50% mortality in *O. surinamensis* adults. Chlorpyrifos was the most toxic insecticide and cypermethrin was the least toxic. In addition to causing mortality, insecticide exposure caused GST enzyme induction in *O. surinamensis* adults, an effect that could play a role in the development of insecticide metabolic resistance. More work is recommended to determine parameters of development of insecticide resistance in *O. surinamensis* populations.

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