Journal of Biopesticides, 3(3): 586 - 589 (2010)

Toxic properties of certain botanical extracts against three major stored product pests

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ABSTRACT

Acetonic extracts of melon pumpkin, *Cucurbita maxima* (Duchesne), Sweet Orange, *Citrus sinensis* (L.) and Sour Orange, *Citrus aurantium* (L.), were evaluated for insecticidal activity against adults of three stored product pests *viz.*, *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (Fabricius) and *Tribolium castaneum* (Herbst), by filter paper diffusion method (direct contact application) and fumigation methods. The insect responses towards the treatment depended on the type of plant, the time of exposure and also the mode of application. In a test with the filter paper diffusion method (contact application), *C. maxima* leaf extract showed 100% mortality to *S. oryzae* and *R. dominica* within three days after treatment (DAT) at the rate of 8.5 mg/cm^2, whereas, only 65% mortality was observed against *T. castaneum* at this dosage. The application of the crude plant extracts, *C. sinensis* and *C. aurantium* in the same concentrations, caused 89 % and 76% mortality to *S. oryzae* and *R. dominica* respectively 72 hrs post treatment. Both the extracts produced comparatively less toxicity to *T. castaneum* and *C. sinensis* within the same duration of time. Among the 3 insects tested *T. castaneum* was the most tolerant having least mortality against all the phytochemicals. The plant compounds lack volatile toxic chemicals as indicated by the absence of significant mortality in fumigation bioassays.

Key words : *Cucurbita maxima*, *Citrus sinensis*, *Citrus aurantium*, toxicity, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, stored product.

INTRODUCTION

Stored-product insects can cause post harvest losses, estimated from 9% in developed countries to 20% or more in developing countries (Phillips and Throne, 2010). The rice weevil, *Sitophilus oryzae* (L.), Lesser Grain Borer, *Rhyzopertha dominica* (Fabricius) and red flour beetle, *Tribolium castaneum* (Herbst), are major pests of stored grain products in the tropics. At first the control of insect pests in storage is largely based on synthetic insecticides and fumigants (pirimiphos methyl and phosphine) which have led to the development of insecticide resistant strains, increasing cost of application, lethal effects on non-target organisms in addition to direct toxicity to users (Champman and Dyte, 1976; Ignatowicz and Wesolowska, 1994; Best and Ruthven, 1995). Also their adverse environmental effects and the need to maintain a sustainable environment have created the need for environmental-safe, degradable and target specific insecticides. Because of this much effort has been focused on plants or their constituents as potential sources of commercial insect control agents (Han *et al.*, 2006). Considerable efforts have been focused on plant-derived materials, potentially useful as commercial insecticides.

The uses of plant materials in pest control become an important alternative to the use of synthetic insecticides (Aranson *et al.*, 1989). Plants are a rich source of the chemical compounds with various medicinal and insecticidal properties (Arnason *et al.*, 1989). The insecticidal activity of many plant derivatives against several stored product pests has been demonstrated (Golob *et al.*, 1999, Weaver and Subramanyam, 2000; Usha Rani and Udaya Lakshmi, 2007). Many plant chemicals have larvicidal, pupicidal and adulticidal activities, most being repellants, ovipositional deterrents and antifeedants against both agricultural pests and medically important insect species. Some of the Citrus species have been reported as a source of botanical insecticides: as a variety of these plants contain secondary metabolites that show insecticidal activity against several coleopteran and dipteran (Su *et al.*, 1972; Abbassy *et al.*, 1979; Greany *et.al.*, 1983; Sheppard, 1984; Salvatore *et al.*, 2004; Shrivastava *et al.*, 2010) and lepidopteran species (Sahayaraj, 1998). Limonoids, extremely bitter chemicals present in citrus seeds, act as antifeedants or antagonize ecdysone action in many lepidopteran species (Klocke and Kubo, 1982, Jayaprakaha *et al.*, 1997).

The present study was undertaken to assess the potential of plant extracts for their insectistatic potential against various stored product pests. In this paper we report the results obtained on biological evaluation of *Cucurbita maxima*, *Citrus sinensis* and *Citrus aurantium* in the form of foliar and peel extracts. The aim in selecting this plants is to identify the plants effective as natural pest control agents that can be grown easily in the farmers residential or cropping area, so that the pesticides of natural origin that are economically viable and environmentally safe are easily available for the users.

MATERIALS AND METHODS

Insects

The cultures of rice weevil *Sitophilus oryzae*, the adzuki bean weevil *Rhyzopertha dominica* and the red flour beetle *Tribolium castaneum*, were maintained in the laboratory without exposure to any insecticide. *S. oryzae, T. castaneum* and *R. dominica* were reared on rice grain, at 28 ± 2 ºC, 55–65% RH, and a 16:8 light: dark photoperiod.

Plant materials and their preparations

The three plants melon pumpkin, *Cucurbita maxima* (DUCH.) (Cucurbitaceae), sweet orange, *Citrus sinensis* (L.), and sour Orange, *Citrus aurantium* (L.), (Rutaceae) were chosen for the experiments and were grown in the fields of Acharya N. G. Ranga agricultural University at Hyderabad, India. The leaf of *C. maxima* (500 g) and the fruit peels of *C. sinensis* (650 g) and *C. aurantium* (600 g) were shade dried and coarsely powdered using an auto mixer. The materials were then extracted twice with 1000 ml acetone at room temperature for 3 days and filtered (Whatman No. 1). The filtrate was concentrated to dryness by rotary evaporation at 55^0 C. The yield of each acetone extraction was *C. maxima* 26.5 g, *C. sinensis* 32.3 g and *C. aurantium* 18.1 g.

Bioassay

The insecticidal activities of the *C. maxima*, *C. sinensis* and *C. aurantium* against test insects were determined by direct contact application. The crude extracts at different concentrations of 100, 90, 80, 70 and 60 mg in 200 ìl acetone was applied on to filter papers (Whatman (No.1) 4.25 cm diameter), which gave a dosage of 8.5, 7.65, 6.80, 5.95 and 5.10 mg/cm^2 respectively. Whereas the control category received 200 µl acetone without any plant products. After drying under a fume hood for 3 min each filter paper was placed in the bottom of a petridish (5 cm diameter × 1 cm) and then ten numbers of 2-5 d old, adults of *S. oryzae*, *R. dominica* and *T. castaneum* were released into the petridish and exposed to treatments. Results on mortality were noted after every 24 hrs of treatment. In a similar experiment, the filter papers treated with acetone alone served as controls. Less than 5% of test insects were in contact with the wall or lid of a petridish at a given time. All the experiments were carried at room temparature 28 ± 2 ºC, 55 - 65% RH, and photoperiod of 16:8 light dark.

In a separate experiment, the responses of *S. oryzae*, *R. dominica* and *T. castaneum*, to the fumigant action of the crude extracts were investigated according to the method of Kim and Ahn (2001). Filter paper discs (4.7cm dia) treated with a concentration of 8.5 mg/cm^2 of the compounds, was placed at the bottom of a polyethylene cup (4.8 cm diameter × 8.6 cm), and a diet cup containing adults was put into the polyethylene cup. This prevented direct contact of the test adults with the test compound. Groups of 10 adults were placed in diet cups (3.8 cm dia x 4 cm) covered with a 60-mesh cloth. Each polyethylene cup was then sealed with a lid. The insects were exposed for 3 days. Mortality counts were made every 24hrs. All treatments were replicated ten times.

Statistical analysis

The percentage mortality was determined and transformed to arcsine square - root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at $P = 0.05$.

RESULTS AND DISCUSSION

The acetonic extracts of *C. maxima* and the peel extracts of *C. sinensis* and *C. aurantium* that produced varied levels of toxic properties to the pest species were examined. In the contact application method, the mortality rates depended on the type of plant extract, exposure time and the dosage. At a dose of 8.5 mg/cm^2 *C. maxima* extract showed 100% mortality against *S. oryzae* and *R. dominica*, whereas it was only 68 ± 1.5% in the case of *T. castaneum*t 3 DAT (Table 1). The peel extracts of *C. sinensis* and *C. aurantium* were somewhat similar in action and produced nearly the same percentage of mortality i.e. 89% and 76% to *S. oryzae* and *R. dominica* at 8.5 mg/cm^2 and 3 DAT. *T. castaneum* is slightly tolerant to most of the treatments as evident from the percentage mortality obtained with *C. sinensis* and *C. aurantium* (Table 1). However, in control category no toxicity was observed. Entirely different results were obtained with the fumigation treatments of the plant crude extracts. The toxicity of the compounds is not significant to all the test insects (Table 2). It appears that the volatile toxicants are not present in significant quantities in all the 3 plant extracts. It is somewhat surprising, as though the plants selected have strong odour, they failed to show any effect on the pest toxicity.

Table 1. Insecticidal activity of *C. maxima*, C. *sinensis* and C.*aurantium* extracts against 3 major stored product pests in contact mode of application 3DAT[a]

Plants	Dose (mg/cm²)	*S.oryzae*	*R.dominica*	*T.castaneum*
C. maxima	5.10	0[a]	0[a]	0[a]
	5.95	27.8 ± 3.3	37.8 ± 3.3	14.4 ± 2.9
	6.80	69 ± 3	80 ± 3.6	40.2 ± 3.9
	7.65	80.4 ± 3.1	82.8 ± 2.3	46.8±3.0
	8.5	100	100	68±1.5
C. *sinensis*	5.10	0[a]	0[a]	0[a]
	5.95	0[a]	5.0 ± 0.3	0[a]
	6.80	20 ± 2.8	35.6 ± 2.8	11.6 ± 1.2
	8.5	89 ± 2.0	75.6 ± 1.7	67.6 ± 2.7
C.*auran-tium*	5.10	0[a]	0[a]	0[a]
	5.95	10.4 ± 1.9	14 ± 1.4	0[a]
	6.80	29 ± 2.3	34 ± 1.5	4.0 ± 0.3
	7.65	61.4 ± 1.9	67 ± 2.6	20.6 ± 0.7
	8.5	88.2 ± 2.0	75.2 ± 1.6	49.4 ± 1.4

[a]Each datum represents the mean of five replicates, each set up with 10 adults (n = 50);[b] Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Scheffe's test).

We conclude that only *C. maxima* crude extract is highly effective against the stored product insects used in the investigations which caused 100% mortality to *S. oryzae* and *R. dominica*. Previously, *C. maxima* seeds were shown to have diversified biological functions, such as insecticidal properties against certain agricultural pests (Zhou *et al.,* 2000). Larvicidal, ovicidal and repellent activities of the leaf extract of *C. maxima* plants against *Culex quinquefasciatus* (Say) (Mullai and Jebanesan, 2007) and also for organic forming (Shrivastava *et al*., 2010) have been reported. *C. sinensis* and *C. aurantifolia* were shown to contain insecticidal activity against mosquito, cockroach and housefly (Ezeonu *et al.*, 2001). Siskos *et al*., 2007 suggested that *C. aurantium* contains secondary metabolites that are toxic to olive fruit fly, *Bactrocera oleae* adults. There are several reports on the insecticidal activity of *C. aurantifolia, C. sinensis* and grapefruit, *C. paradisii*, peel oils against *T. confusum* J. and *S. granarius* L. (Abbassy *et al.*, 1979). However, the present data suggests that the acetone extracts of the peels of *C. sinensis* and *C. aurantium* were effective against S. *oryzae, R. dominica* and *T. castaneum*. Govindan and Nelson (2009) suggested *Azadirachta indica* A. Juss. and *Alpinia officinarum* Hance. for the management of *S. oryzae*.

Table 2. Insecticidal activity of *C. maxima*, *C. sinensis* and *C. aurantium* extracts against three major stored product pests in contact mode of application 3DAT[a]

Plants	Dose (mg/cm²)	*S.oryzae*	*R.dominica*	*T.castaneum*
C. maxima	8.5	18.3 ± 03	21 ± 0.5	0[a]
C. *sinensis*	8.5	14 ± 0.5	17.6 ± 0.3	0[a]
C. *aurantium*	8.5	5.6 ± 0.3	13 ± 0.5	0[a]

[a]Each datum represents the mean of five replicates, each set up with 10 adults (n = 50); [b]Means within a column followed by the same letter are not significantly different at $P = 0.05$ (Scheffe's test).

From these results it was concluded that the plants *C. maxima*, *C. sinensis* and *C. aurantium* exhibits insecticidal activity to *S. oryzae*, *R. dominica* and *T. castaneum*. However, these plants did not show much impact on *T. castaneum* than other two insects studied. Further analysis to isolate the active compounds for insect control is under way in our laboratory.

ACKNOWLEDGEMENT

The authors are grateful to Dr. J. S. Yadav, Director of Indian Institute of Chemical Technology, Hyderabad, for the facilities and encouragement.

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Received: October 14, 2009 Revised: July 7, 2010 Accepted: July 19, 2010