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Insecticidal and repellent activities of the chloroform extracts of *Urena sinuata* L. against *Tribolium castaneum* (Herbst) adults

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Abstract: The insecticidal and repellent activity tests of the chloroform extracts of fruit, leaf, root and stem of *Urena sinuata* L. against the red flour beetle *Tribolium castaneum* L. adults have been conducted. The leaf, root and stem extracts showed high toxicity by giving mortality of the beetles within 30 min. of exposure. The LD_{50} values for the 12, 24, 36 and 48h of exposures were 0.354, 0.262, 0.209 and 0.196mg cm^2 for the fruit extract; 0.587, 0.413, 0.355 and 0.299mg cm^2 for the leaf extract; 0.440, 0.389, 0.340 and 0.268mg cm^2 for the root extract and 0.968, 0.661, 0.491 and 0.362mg cm^2 for the stem extract. The insecticidal activity can be arranged in the order of fruit> root> leaf> stem extracts. The root and stem showed repellent activity against *T. castaneum* adults at $P<0.01$ and $P<0.05$ levels of significance, while the fruit and the leaf extracts did not show repellency at all.

Keywords: Mortality, repellency, *Urena sinuata, Tribolium castaneum.*

Introduction

Urena sinuata L. (Family Malvaceae) is a wild shrubby plant with some folk medicinal use in its native areas. It has many synonyms, i.e. *U. lobata, U. morifolia, U. moricata, U. paradoxa* and *U. swartzii.* Various species of this plant grow in different tropical and sub-tropical areas throughout the world. The roots are sweet, slightly cooling, antirheumatic and antipyretic. A decoction of the stem and roots is used in Brazil as a remedy in severe windy colic. A poultice prepared from the roots and leaves is used as an emollient. The flowers are administered as a pectoral and expectorant in dry and inveterate coughs. An infusion of the flowers is used as a gargle for aphthae and sore throat. An infusion of the flower is used in bronchitis (Kirtikar and Basu, 1965). In India the root is used as an external application for lumbago. It is used for reproductive purposes in the Pacific, Trinidad and Tobago, China and India (Browner, 1985), while Cheryl (2007) mentioned that it is used for specific human problems of both the genders. It is a popular diuretic in Assam, and also used as an abortifacient. In the Philippines the root is considered emollient, refrigerant and maturant; the leaves are prescribed in inflammation of the intestines and the bladder. A decoction of dried roots is used in enteritis, dysentery, rheumatic pains and tonsillitis. A poultice of fresh leaves is given for snake bites, sprains and bruises. Anyway, some view the plant as a medicinal plant, some view as a weed, but others make use of its fiber (Aramina fiber) for various purposes in Madagscar, Nigeria and Western Sudan, Chad, Central African Republic, Zaire and Gabon, which is said to resistant to damage by termites and water (Anon, 1976; Ahmed *et al.,* 2009). However, information on its biological activity, as well as of phytochemical information is still scanty. This is why the plant was taken into consideration for investigation of its bioactive potentials.

Materials and Methods

***Collection and preparation of test materials*:** Fresh plants (*U. sinuata*) were collected from the Rajshahi University Campus, Rajshahi-6205, Bangladesh. Its identification was confirmed by Prof. ATM. Naderuzzaman, and a voucher specimen (No. 41, 02-04-2008) is also kept in the herbarium of the Department of Botany, University of Rajshahi. After collection leaves, roots and stem were separated, and were chopped into small pieces to dry in a well-ventilated room/under shade. Fruits were also collected and processed. Dried materials were then powdered in a grinder, weighed and placed in separate conical flasks to add chloroform (100 gm × 300 ml × 3 times) for 48 hours. Filtration was done by Whatman filter paper at 24h interval in the same flask followed by evaporation until the extract was left. It was then removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

***Collection and culture of the test insect*:** *Tribolium castaneum* used in the present experiment was reared in glass beakers (500 ml) in a standard mixture of whole-wheat flour with powdered dry yeast (19:1) (Park, 1962; Zyromska-Rudzka, 1966) in an incubator at 30°C ±0.5°C without light and humidity control for continuous supply of adults.

***Dose-mortality test against T. castaneum*:** For dose-mortality responses by surface film method doses of the $CHCl_3$ extracts of fruits, leaves, roots and stem were selected through *ad hoc* experiments

by putting 50mg of each of the extracts diluted separately in 1ml of solvent to apply in 50mm Petri dishes and by increasing or decreasing the amount of extracts in repeated manner until a suitable mortality range was obtained. The doses selected for the fruit extract were 0.255, 0.191, 0.127, 0.096 and 0.064mg/cm^2, for the leaf extract 1.529, 0.764, 0.382, 0.191 and 0.096mg/cm^2, for root extract 1.019, 0.510, 0.255, 0.127, 0.064mg/cm^2 and for stem extract 3.822, 1.911, 0.955, 0.478 and 0.239mg/cm^2. Each of the five doses were diluted in 1ml of solvent, poured into a Petri dish and allowed to dry. Ten adult beetles were released in each Petri dish, and the experiment of all the doses for each of the extracts was set in 3 replicates. The mortality of the beetles was assessed at certain intervals.

***Statistical analysis of the dose-mortality data*:** The mortality of the beetles was observed after 12, 24, 36 and 48h post exposure. However an instant observation was made just after 30 min. of application for the detection of acute toxicity, if any. The mortality (%) was corrected by Abbott's formula (1925).

$$P_r = \frac{P_0 - P_c}{100 - P_c} \times 100$$

Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), and P_c = Control mortality (%). The statistical analyses were done according to Finney (1947) and Busvine (1971) to calculate the LD_{50} values.

Repellent activity test against *T. castaneum* adults: The methodology for repellency test used in the experiment was adopted from the method (No. 3) of McDonald *et al*. (1970) with some modifications by Talukder and Howse (1993, 1994). Half filter paper discs (Whatman No. 40, diameter 9 cm) were treated with the selected doses of 0.079, 0.039, 0.020, 0.010 and 0.005mg cm^2 of fruit extract and were then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in the Petri dishes. The orientation was changed in the 2 remaining replicates to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Ten adult insects were released in the middle of each of the filter paper circles. The same was then done for the leaf, root and stem extracts.

***Observation and analysis of repellency data*:** Each concentration was tested for five times. Insects that settled on each of the non-treated half of the filter paper discs were counted after 1h and then observed repeatedly at hourly intervals for five hours. The average of the counts was converted to percent repellency (*PR*) using the formula of Talukder and Howse (1993, 1995): *PR* = 2 (C-50), where, C is the percentage of insects on the untreated half of the disc.

Results and Discussion

Dose mortality effects: The results of the dose-mortality assay of fruit, leaf, root and stem extracts are represented in Table 1. For 12, 24, 36 and 48h of exposures the LD_{50} values for the fruit extract ranged between 0.354 to 0.196 mg/cm^2; for the leaf extract between 0.587 to 0.299mg/cm^2; for the root extract between 0.440 to 0.268mg/cm^2 and for the stem extract the values ranged between 0.968 to 0.362mg/cm^2. The insecticidal activity could be arranged as fruit> root> leaf> stem extracts. Except the fruit extract the leaf, root and stem extracts showed acute toxicity by giving mortality of the beetles within 30min. of exposure.

Table 1. LD_{50} values of fruit, leaf, root and stem extracts ($CHCl_3$) of *U. sinuata* against *T. cstaneum* adults.

Plant parts	Duration of exposure				
	30 min.	12h	24h	36h	48h
Fruit	-	0.345	0.262	0.209	0.196
Leaf	3.807	0.587	0.413	0.355	0.299
Root	1.406	0.440	0.389	0.340	0.268
Stem	4.516	0.968	0.661	0.491	0.362

Repellent effects: The repellency results are presented in Tables 2 and 3. The $CHCl_3$ extracts of the root and stem of *U. sinuata* offered a promising repellent effect against *T. castaneum* adults ($P<0.01$ and $P<0.05$ respectively) while the fruit and the leaf extracts did not show any repellency in the beetles.

These findings receive supports from previous researchers' achievements. Mazumder *et al.* (2001) found a broad spectrum antibacterial activity of *Urena lobata* root extract; and the same was supported by Hernández *et al.* (2003). A report on cytotoxic effect of *U. sinuata* leaf extractives has been made very recently (Sosa and Rosquete, 2010), while another report reveals that the leaves of this plant contain urease and an unidentified alkaloid (Anon, 1976), and alkaloids normally show biological activity.

Table 2. Percent repulsion values and the arcsin transformed data of the fruit, leaf, root and stem extracts ($CHCl_3$) of *U. sinuata* against *T. castaneum* adults.

Plant part	Dose (mg/cm^2)	Percent repulsion PR = (Nc – 5)×20 (at regular intervals)				
		1h	2h	3h	4h	5h
Fruit	10	26.66 (31.05)	40 (39.23)	6.66 (14.89)	33.34 (35.24)	26.66 (31.05)
	5	-13.34 (21.39)	-6.66 (14.89)	13.34 (21.39)	13.34 (21.39)	40 (39.23)
	2.5	13.34 (21.39)	13.34 (21.39)	-6.66 (14.89)	20 (26.57)	-46.66 (43.05)
	1.25	-13.34 (21.39)	-40 (39.23)	-86.66 (68.53)	-73.34 (58.89)	-40 (39.23)
	0.625	13. (21.39)	-20 (26.57)	-20 (26.57)	6.66 (14.89)	6.66 (14.89)
Leaf	10	0 (0.00)	-6.66 (14.89)	-6.66 (14.89)	6.66 (14.89)	0 (0.00)
	5	-20 (26.57)	-13.34 (21.39)	-13.34 (21.39)	-33.34 (35.24)	-40 (39.23)
	2.5	6.66 (14.89)	-6.66 (14.89)	-6.66 (14.89)	13.34 (21.39)	-13.34 (21.39)
	1.25	-6.66 (14.89)	-26.66 (31.05)	13.34 (21.39)	6.66 (14.89)	-26.66 (31.05)
	0.625	6.66 (14.89)	-26.66 (31.05)	-60 (50.77)	-13.34 (21.39)	-26.66 (31.05)
Root	10	46.66 (43.05)	66.66 (54.70)	73.34 (58.89)	86.66 (68.53)	100 (84.84)
	5	-6.66 (14.89)	26.66 (31.05)	33.34 (35.24)	13.34 (21.39)	40 (39.23)
	2.5	60 (50.77)	60 (50.77)	73.34 (58.89)	80 (63.43)	73.34 (58.89)
	1.25	-20 (26.57)	33.34 (35.24)	33.34 (35.24)	0 (0.00)	26.66 (31.05)
	0.625	13.34 (21.39)	26.66 (31.05)	13.34 (21.39)	20 (26.57)	33.34 (35.24)
Stem	10	-53.34 (46.89)	-73.34 (58.89)	-80 (63.43)	-100 (84.84)	-93.34 (75.00)
	5	-33.34 (35.24)	-26.66 (31.05)	-26.66 (31.05)	-53.34 (46.89)	-46.66 (43.05)
	2.5	13.34 (21.39)	0 (0.00)	-26.66 (31.05)	-33.34 (35.24)	-40 (39.23)
	1.25	0 (0.00)	-46.66 (43.05)	-66.66 (54.70)	-86.66 (68.53)	-66.66 (54.70)
	0.625	6.66 (14.89)	6.66 (14.89)	13.34 (21.39)	-33.34 (35.24)	-40 (39.23)

Table 3. Repellency effect of fruit, leaf, root and stem extracts ($CHCl_3$) of *U. sinuata* against *T. castaneum* adults.

Extract	Between doses (df =4)		Between time interval (df=4)	
	F-value	Level of significance	F-value	Level of significance
Fruit	0.500	-	3.236	-
Leaf	1.205	-	4.897	-
Root	2.446	-	16.105**	P<0.01
Stem	6.994	-	11.576*	P<0.05

The juice of the leaves and roots of this plant is widely used in the treatment of various bowel complaints (Ahmed *et al.,* 2009). In India the root is used as an external application for lumbago (Kirtikar and Basu, 1965) and also as external remedy for rheumatism (Watt, 1972). For the control of stored product pests use of plant extract in laboratory trials is no more something new now-a-days. Khan *et al.* (1998) and Khatun *et al.* (1999) showed insecticidal activities of *Thevetia peruviana* seed and leaf extracts respectively. Insecticidal and repellent potentials of spice extracts against *Tribolium castaneum* adults were shown by Islam *et al.* (2004) for *Cinnamomum zeylanicum, Sygygium aromaticum* & *Myristica fragrans*; and by Farhana *et al.* (2006) for *Coriandrum sativum, Trachyspermum ammi* & *Trigonella foenum-graecum*. Parveen *et al.* (2007) revealed nut shell, root bark and stem bark of *Anacardium occidentale* L. as sources of insecticidal properties against *Tribolium castaneum*. A perusal of the data achieved in this experiment clearly showed the presence of insecticidal properties in *U. sinuata,* as well as traces of repellent potential. Thus, a comprehensive phytochemical analysis of the plant for its insecticidal and repellent components, as well as the physiological studies of the active ingredients are very much to be solicited for their effective use in the future pest control and pharmaceutical endeavors.

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