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Pesticidal and pest repellency activities of roots of *Laportea crenulata* (Gaud.) against *Tribolium castaneum* (Herbst)

A. Khan[1, 5]*, M. Hedayetul Islam[2], M. A. Alim Al-Bari[1], M. Ekramul Islam[1], M. S. Parvin[1], T. Zaman[3], M. Nurul Islam[4] and M. E. Haque[1]

[1]*Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh*
[2]*Institute of Food and Radiation Biology, AERE, Savar, Dhaka-1349, Bangladesh*
[3]*Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh*
[4]*Department of Zoology, University of Rajshahi, Rajshahi, Bangladesh*
[5]*Department of Metabolism and Aging, The Scripps Research Institute, Florida, USA*
E-mails: alamkhan792002@yahoo.co.in & akhan@scripps.edu

Abstract

The presence of *Tribolium castaneum* (Herbst) in a stored food results in contamination and substantial economic damage due to loss of the products and a decrease in nutritional value. This study was conducted to determine the pesticidal and pest repellent activities of roots of *Laportea crenulata* Gaud. against *T. castaneum*. Pesticidal activity was determined using surface film method, whereas, filter paper disc method was used to determine the pest repellency property of the plant. Both chloroform and methanol soluble fractions of ethanol extracts of roots of L crenulata were found to possess pesticidal property. High mortality record (77-83% at 24-48 hours exposure duration) for chloroform fraction was found at a dose 1.77mg/cm^2. On the other hand, methanol fraction showed high mortality records (80-93%) at doses 0.44-1.77 mg/cm^2, suggesting its superior pesticidal potency. Although, both fractions also showed pest repellency property, chloroform fraction has higher activity than methanol fraction. These results suggests that roots of *L. crenulata* possess both pesticidal and pest repellency activities against *T castaneum* and can be used in controlling pest of grain and flour based products.

Keywords*: Laportea crenulata*; ethanol extract; methanol fraction; chloroform fraction; *Tribolium castaneum*

1. Introduction

Tribolium castaneum (Herbst) is a major pest/insect of stored grain and flour-based products in all tropical and subtropical countries of the world. A wide range of stored food commodities including grain, flour, peas, beans, nuts, dried fruits and spices were affected by *T. castaneum* (Pugazhvendan et al., 2009). Their presence in a stored food results in contamination and substantial economic damage due to loss of the products and a decrease in nutritional value. A number of synthetic agents (e.g. methoprene, permethrin, cypermethrin, deltamethrin and fenvalerate etc) were identified for good activity against *T. castaneum*, however, use of these agents has led to problems such as environmental disturbances, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms, in addition to direct toxicity to users (Isman, 2006). Dyte and Blackman (1972) reported that almost all of the strains of *T. castaneum* have become resistant to malathion. To minimize the use of synthetic pesticides and to avoid environmental pollution, natural pesticide and repellent substances have been searched for pest control during recent times (Govindachari et al., 2000). Plant products having considerable pesticidal potential are gaining tremendous importance in recent years because such products minimizes disadvantages associated with synthetic agents (Pugazhvendan et al., 2012).

Laportea crenulata Gaud. (Synonym *Urtica crenulata* Roxb.) is an evergreen shrub of Urticaceae family (Khan et al., 2011). The *Urticaceae* are monoecious or dioecious herbs or infrequently shrubs or small trees comprising 45 genera and 700 species, often with specialized stinging hairs (Khan et al., 2008). About 9 genera and 60 species are available in Bangladesh (Hasan and Haque, 1993). In Bangladesh *L. crenulata* is locally known as Agnichutra, and is also distributed in India and the Malay islands (Khan et al., 2011). The roots are used traditionally for the treatment of bleeding from nose and/or mouth, excessive gas in the stomach, constipation, weakness, asthma, gout, mumps, whooping cough, and chronic fever

*Corresponding author
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(Rahman et al., 2008). The roots of the plant also have stimulant, stomachic and diuretic properties (Rahman et al., 2008). In searching botanical agent(s) to control *T. castaneum*, the present study was aimed to determine the pesticidal and pest repellency activities of the roots of *L. crenulata* against *T. castaneum*.

2. Materials and methods

2. 1. Plant materials

The fresh roots of the plant were collected in the month of October to November from various parts of Rangpur district of Bangladesh (yr). The plant was taxonomically identified by Professor A. T. M. Naderuzzaman, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh and its voucher specimen (No. 1239) was deposited.

The roots were first washed with water to remove the adhering dirt, cut into small pieces, sun dried for three days and finally dried at 45^0C for 36 h in an electrical oven (Khan et al., 2007a). After complete drying, the entire portions were pulverized into a coarse powder (Pandey and Brave, 2011) with the help of a grinding machine (FFc-15, China) and were stored in an air tight container for further use.

2. 2. Extraction of plant materials

Powdered dried roots (900 g) of the plant were extracted (cold) with ethanol (5L) in flat bottom glass containers, through occasional shaking and stirring for 10 days. The whole extract was filtered and the solvent was evaporated to dryness in vacuo with an rotary evaporator at 40-50°C to afford a blackish green mass (45 g), which was further extracted with petroleum ether (3x50 mL), chloroform (3x50 mL) and methanol (3x50 mL) to afford petroleum ether (3 g), chloroform (7 g), and methanol (9 g) fractions, respectively (Jeffery et al., 2000, Khan et al., 2007b).

2. 3. Collection and maintenance of pest

T. castaneum (Herbst) used in the present experiment was originally received from the Crop Protection Department of the University of Newcastle upon Tyne, U.K. and were reared in the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. The pest was maintained in 1 L glass jar containing food medium. A filter paper was placed inside each jar for easy movement of the pest. The jar was covered with a filter paper at the top, and kept in an incubator at 30 ± 0.5^0C. Wheat flour and powdered brewers yeast in the ratio of 19: 1 was used as food medium to culture the pest. Both flour and yeast were previously passed through a 250 micrometer aperture sieve and mixed thoroughly using an electric blender. The food was sterilized in an oven at 120^0C for 6 h. Food was not used until at least 15 d after sterilization to allow its moisture content to equilibrate with that of environment.

2. 4. Screening for pesticidal activity

Pesticidal activity was determined using surface film method (Farhana et al., 2006; Iram et al., 2013), as stated below.

Preparation of working solution: To prepare the working solution 100 mg experimental sample was dissolved in 2 mL mixed solvent (50% chloroform + 50% methanol) in a vial. For each sample three similar vials were prepared.

Preparation and application of doses: Thirteen clean and dried petridishes (60 mm, size, 28.26 cm^2 area) were taken for each sample. Four petridishes were marked by 50 mg, 25 mg, 12.5 mg and 6.25 mg. One mL working solution (prepared previously) was poured into the 50 mg petridish and agitated clockwise, anticlockwise, left to right and right to left to further confirm the uniform dispersion. 1 mL solvent (50% chloroform + 50% methanol) was added to that vial from which 1 mL had been used and mixed uniformly. From this vial, 1 mL solution was poured into the 25 mg petridish and agitated similarly for uniform dispersion. Using this serial dilution technique, the sample was likewise poured into 12.5 mg and 6.25 mg petridishes and agitated similarly for uniform dispersion. The above processes were continued two times further using two remaining vials of working solution and eight remaining petridishes. Then the layers of dispersed sample into the petridishes were air dried. 1 mL solvent (50%chloroform + 50%methanol) was poured and dispersed into control petridish and air dried.

Application of pests and recording of mortality of pests: The pests were collected by sieving and ten pests were applied on each layer of dispersed sample into the petridish. This process is continued for each petridish. Then the number of pests that died were recorded after 24 h and 48 h.

2. 5. Pest repellency test

Pest repellency test was conducted by filter paper disc method (Farhana et al., 2006; Mondal et al., 2012: Iram et al., 2013), as follows:

Preparation of working solution: The working solution was prepared by dissolving 60 mg experimental sample in 2 mL mixed solvent (50%

chloroform + 50% methanol) in a vial. For each sample three similar vials were prepared.

Preparation and application of doses: Nine clean and dried petridishes (size of each is 90 mm) and nine filter papers (size-90 mm) were taken for each sample. Three petridishes were marked by 30 mg, 15 mg and 7.5 mg. Three filter papers were taken for these three petridishes and each filter paper was cut (by scissors) into two equal parts through the center where one part can be used as control and the other part can be used as treated part. For 30 mg petridish with its filter paper, treated part of filter paper was taken at outer background of the petridish and one mL working solution (prepared previously) was dispersed uniformly throughout this part of filter paper and air dried. Then this part of filter paper was joined with its control part using transparent adhesive tape and placed into the 30 mg petridish using forceps. For 15 mg petridish with its filter paper, treated part of filter paper was taken at outer background of 15 mg petridish. 1 mL solvent (50% chloroform + 50% methanol) was added to that vial from which 1 mL had been used and mixed uniformly. From this vial, 1 mL solution was dispersed uniformly throughout the treated part of filter paper and air dried. Then this part of filter paper was joined with its control part using transparent adhesive tape and placed into the 15 mg petridish using forceps. Similar work was done for 7.5 mg petridish with its filter paper. The above processes were continued two times further using two remaining vials of working solution and six remaining petridishes and filter papers.

Application of pests and recording of repelled pests: Ten pests were applied on the filter paper at the center of the petridish. This process was continued for each petridish. Then the number of pests repelled were counted per one hour interval up to 5 h. The percentages of repellency were determined and results were provided through ANOVA after transforming them into arcsin percentage value.

2. 6. Statistical analysis

The dose-mortality relationship was expressed as a median lethal dose (LD50) using statistical probit analysis (Busvine, 1971). The repellency values in the recorded data were calculated for percent repellency, which was again transformed by arcsine transformation for the calculation of analysis of variance (ANOVA). Means values were compared using one way ANOVA.

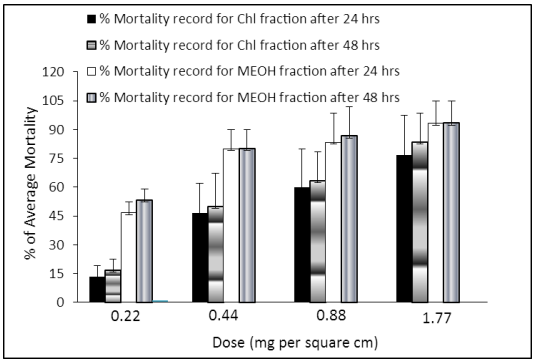

Fig. 1. Mortality records of chloroform (Chl) soluble fraction and Methanol (MEOH) soluble after 24 and 48 hours. Standard deviations are shown as error bar at the top of each column

Table 1. Screening of pesticidal activity for chloroform and methanol soluble fractions after 24 and 48 hours of exposure

Type of Sample	Dose (mg/cm^2)	#	Mortality record for applied pests			
			Record after 24 hs	Av ± SD record after 24 hs	Record after 48 hours	Av ± SD record after 48 hs
Chloroform soluble fraction	1.77	10	10		10	
	1.77	10	6	7.66 ± 2.08	7	8.33 ± 1.52
	1.77	10	7		8	
	0.88	10	8		8	
	0.88	10	6	6 ± 2.00	6	6.33 ± 1.52
	0.88	10	4		5	
	0.44	10	6		6	
	0.44	10	5	4.66 ± 1.52	6	5 ± 1.73
	0.44	10	3		3	
	0.22	10	2	1.33 ± 0.57	2	
	0.22	10	1		2	1.66 ± 0.57
	0.22	10	1		1	
Methanol soluble	1.77	10	10	9.33 ± 1.15	10	9.33 ± 1.15

fraction	1.77	10	10		10	
	1.77	10	8		8	
	0.88	10	10		10	
	0.88	10	8	8.33 ±1.52	9	8.66 ± 1.52
	0.88	10	7		7	
	0.44	10	9		9	
	0.44	10	8	8 ± 1.00	8	8 ± 1.00
	0.44	10	7		7	
	0.22	10	5		6	
	0.22	10	4	4.66 ± 0.57	5	5.33 ± 0.57
	0.22	10	5		5	
	Control	10	0	0	0	0

= Number of pests applied per petridish, Av = Average, hs = hours, SD = standard daviation

3. Results

Both chloroform and methanol soluble fractions have shown pesticidal activity against the *T. castaneum*. However, methanol soluble fraction was found more active than chloroform soluble fraction (Fig. 1). For both fractions, mortality record was increased along with the dose of fraction (Table 1), suggesting the dose dependency for pesticidal activity. At 24 hours of exposure, chloroform soluble fraction has exerted significantly high mortality record (76.66%) only at the high dose (1.77 mg/cm^2), whereas methanol soluble fraction at doses 0.22, 0.44 and 1.77 mg/cm^2 exerted high mortality records 80.00, 83.33 and 93.33%, respectively (Table 2). At 24h of exposure, median lethal doses (LD_{50}) for chloroform and methanol soluble fractions were 0.65 and 0.20 mg/cm^2, respectively. When exposure duration was increased (24 to 48 hours), the mortality records in most cases were minutely increased (Table 1; Fig. 1). Due to the lack in amount of petroleum ether fraction, we did not perform pesticidal and pest repellency experiments for the fraction.

Like pesticidal activity, pest repellency property was also found for both fractions. Pest repellency activity also increased with dose of both fractions. High pest repellency activity for chloroform soluble fraction was observed at 0.47 and 0.94 mg/cm^2 (Table 3a,). Methanol fraction showed good pest repellency activity only at high dose (0.94 mg/cm^2) (Table 3b). At other doses, both fractions showed moderate pest repellency activity. At our first hour of observation (at first observation hour), pest repellency activity of chloroform fraction was weak, which was abruptly increased at subsequent time (2 h) (Table 3a). From second to fifth hours, pest repellency records for both fractions were slightly varied (Table 3a, 3b; Fig. 2).

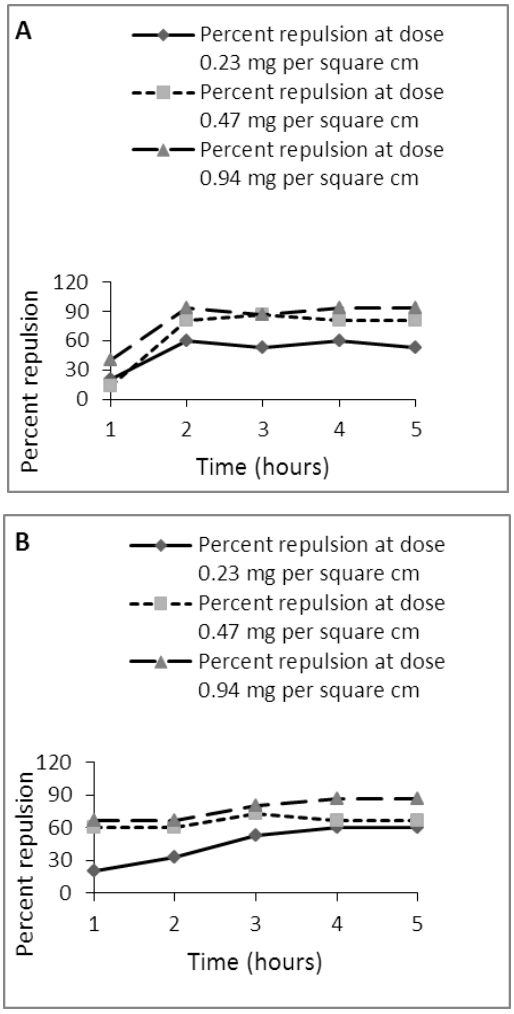

Fig. 2. Repellency records of (A) chloroform and (B) methanol soluble fractions per hour interval up to 5 hours

4. Discussion

Pests/insects often cause extensive damage to stored grain products, which is a serious problem throughout the world (Busvine, 1971). *T. castaneum* is a major paste of most grain and flour based products (Pugazhvendan et al., 2009). Although synthetic pesticides/insecticides are commonly used to control pests, they are now causing concern because of environmental hazards, pests resistance and they are toxic to mammals. In recent years there has been increasing information on the use of alternative methods (Blunt et al., 2005). In many areas of the world, locally available materials are used to protect stored products against damage by pest infestation. Pesticides of plant origin, because of their high degree of tolerance by the mammals, are particularly desired for application against pests of fodders, fruits, vegetables and stored grains (Adeyemi, 2010). Moreover, interest in botanical pesticides has increased as a result of environmental concerns, and pest populations becoming resistant to conventional chemicals. The use of plant extracts in pest control has been practiced for at least two millennia, when botanical pesticides were considered important products for pest management in Ancient China, Egypt, India, Greece (Long et al., 2006; Isman 2006). Jacobson (1989) and Ketkar et al. (1976) have reviewed the effectiveness of plant derivatives for use against grain pests.

Table 2. LD_{50} calculation for pesticidal activity using probit analysis

Sample Type	Recording time	Dose (mg/cm^2)	% of Average Mortality	% Corrected Mortality	Regression equation	LD_{50} (mg/cm^2)	95% Confidence Limits	
							Lower	Upper
Chloroform soluble fraction	After 24 hours	1.77	76.66	77	Esimate 1	0.65	11.03	30.73
		0.88	60	60	Y = 2.48 + 1.97X			
		0.44	46.66	47	Esimate 2			
		0.22	13.33	13	Y = 2.60 + 1.89X			
	After 48 hours	1.77	83.33	83	Esimate 1	0.56	9.66	25.75
		0.88	63.33	63	Y = 2.58 + 2.00X			
		0.44	50.00	50	Esimate 2			
		0.22	16.66	17	Y = 2.60 + 1.99X			
Methanol soluble fraction	After 24 hours	1.77	93.33	93	Esimate 1	0.20	2.13	15.88
		0.88	83.33	83	Y = 3.81 + 1.58X			
		0.44	80.00	80	Esimate 2			
		0.22	46.66	47	Y = 3.75 + 1.62X			
	After 48 hours	1.77	93.33	93	Esimate 1	0.16	1.33	16.14
		0.88	86.66	87	Y = 4.02 + 1.48X			
		0.44	80.00	80	Esimate 2			
		0.22	53.33	53	Y = 3.98 + 1.52X			

In the present investigation, both chloroform and methanol soluble fractions of ethanol extract of roots of *L. crenulata* were found to possess pesticidal property against *T. castaneum*. Repellency activity against T castaneum was also found for both fractions. Control of pest in stored food products can be achieved by pesticidal and pest repellency activities (Farhana et al., 2006; Mondal et al., 2012; Iram et al., 2013)

Table 3a. Pest repellency record and percent repulsion (PR) for chloroform ext. of *Laportea crenulata* Gaud

Dose (mg/cm^2)	#	Repellency record													
		Hourly observation					Average of hourly Observation (Nc)					Percent repulsion (PR) PR=(Nc-5)x20%			
		1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h
0.94	10	7	10	10	10	10										
0.94	10	8	9	9	10	9	7.00	9.66	9.33	9.66	9.66	40%	93.2%	86.6%	93.2%	93.2%
0.94	10	6	10	9	9	10										
0.47	10	6	9	9	9	9										
0.47	10	5	10	9	8	8	5.66	9.00	9.33	9.00	9.00	13%	80%	86.6%	80%	80%
0.47	10	6	8	10	10	10										
0.23	10	6	8	8	9	8										
0.23	10	6	9	8	8	8	6.00	8.00	7.66	8.00	7.66	20%	60%	53.2%	60%	53.2%
0.23	10	6	7	7	7	7										

Table 3b. Pest repellency record and percent repulsion (PR) for methanol soluble fraction of *L. crenulata* Gaud

Dose (mg/cm^2)	#	Repellency record														
		Hourly observation					Average of hourly Observation (Nc)					Percent repulsion (PR) PR=(Nc-5)x20%				
		1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h
0.94	10	7	8	9	10	10										
0.94	10	9	8	9	9	9	8.33	8.33	9.00	9.33	9.33	66.6%	66.6%	80%	86.6%	86.6%
0.94	10	9	9	9	9	9										
0.47	10	7	7	8	9	9										
0.47	10	9	8	8	8	7	8.00	8.00	8.66	8.33	8.33	60%	60%	73.2%	66.6%	66.6%
0.47	10	8	9	10	8	9										
0.23	10	6	7	8	8	8										
0.23	10	6	7	7	8	8	6.00	6.66	7.66	8.00	8.00	20%	33.2%	53.2%	60%	60%
0.23	10	6	6	8	8	8										

= Number of pests applied, h = hour

Methanol fraction has shown higher pesticidal potency than chloroform fraction (Table 1; Fig. 2), however, regarding pest repellency property, chloroform fraction was found to have higher potency (Table 3a, 3b). This might be due to different composition of chloroform and methanol soluble fractions as well as volatility nature of fractions. In spite of the wide-spread recognition that many plants possess pesticidal properties and minimizes disadvantages associated with synthetic pesticides, only a small number of pest control products are directly obtained from plants (Isman, 1997; 2000). Botanicals used as pesticides presently constitute only 1% of the world pesticide market (Rozman et al., 2007). Because of good pesticidal and pest repellency activities, roots of *L. crenualta* hope provide an additional advantage as botanical pesticide in controlling *T. castaneum* of stored food commodities. However, further studies are necessary to isolate compound(s) responsible for pesticidal and pest repellency activities as well as toxicological investigation.

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