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Effect of Bishkatali, *Polygonum hydropiper* L. plant extracts against the red flour beetle, *Tribolium castaneum* Herbst

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Abstract : Toxicity, repellency and residual effects of Bishkatali plant extracts in chloroform and ethyl alcohol solvents were evaluated against the red flour beetle. Five concentrations viz. 500, 250, 125, 62.5 and 31.25mg/ml of Bishkatali plant extracts of both solvents were used in the experiment. The plant extracts in both solvents were moderately toxic to *Tribolium castaneum*. The toxicity of ethyl alcohol extract was more than chloroform extract after 24 and 72 hours treatment on the insect. Bishkatali plant extract in both solvents showed strong repellency against *T. castaneum* in which chloroform extract was better than ethyl alcohol extract. The rate of repellency was increased with the increment of concentration. Both the extracts have produced remarkable residual effect in reducing the progeny of *T. castaneum*. The lowest numbers of F1 adult progeny (32.7, 25.3 and 27.0) emerged from the wheat flour treated with 500mg/10g chloroform extract when parent released at 7, 12, 17 days after treatment respectively. Whereas with 500mg/10g ethyl alcohol extract, 38.0, 29.7 and 30.3 F1 adult progeny emerged when parents released at 7, 12, 17 days after treatment respectively. Bishkatali plant extracts in both chloroform and ethyl alcohol had remarkable residual effects on *T. castaneum* by reducing the production of F1 progeny and/or by increasing the population mortality.

Key words: Botanical, solvent, red flour beetle, mortality, progeny, repellency, residual effect

Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is common and most destructive pest throughout the world. It is generally found in granaries, mills, warehouses, feeding on wheat flour, atta, suji, rice (both husked and unhusked) etc. Neither larva nor adult can generally damage sound grains but they feed on those grains only, which have already been damaged by other pests. This pest has also been reported to attack the germ part (embryo portion) of the grain. Their presence in stored foods directly affects both the quantity and quality of the commodity (Mondal, 1994). In tropical countries like Bangladesh, the climate and storage conditions are favorable for insect growth and development (Talukder & Howse, 1995). The world's cereal production is lost up to 10% every year due to insect infestation in storage (Wolpert, 1967). Today, losses from storage pests cannot be measured only by the amount of food or seeds destroyed by insects. The mere presence of insect fragments in food is objectionable to most consumers. Thus economic losses may be result from insect contamination, although actual losses of food materials due to insect feeding may be negligible.

In the rural areas of South Asia, including Bangladesh farmers traditionally mix leaves, barks, seeds, roots or oils of certain plants with stored grains to keep them free from insect attacks. Such techniques have been inherited as part of the traditional culture (Saxena *et al.,* 1988). The earlier studies (Talukder & Howse, 1993; 1994 and 1995) have established the successful effects of different plant parts and or extracts against different major stored products insect pests in Bangladesh. There are about 2000 plants have been reported to possess pest control properties (Ahmed *et al.,* 1984). Moreover, products from several floral species have been demonstrated to act as repellents, toxicants and antifeedants against a number of Coleoptera that attack stored products (Rahman & Schmidt, 1999; Raja *et al.,* 2001; Tapondjou *et al.,* 2002). Khanam *et al.* (2006) also reported toxic and repellent properties of sugarcane bagasse-based lignin against some stored grain insect pests including *T. castaneum.*

However, few works has been done in Bangladesh to determine the efficacy of our locally available plant materials against stored products insect pests. Thus an investigation was undertaken to determine the toxicity, repellency and residual effects of the Bishkatali, *Polygonum hydropiper* Linn. plant extracts on the red flour beetle, *T. castaneum*.

Materials and Methods

Experiments were conducted in the Laboratory, Department of Entomology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period of March 01 to August 31, 2006. Adult beetles were collected from the laboratory mass culture and reared in glass beaker (500ml) with standard food medium (wheat flour: dry yeast 19:1) in an incubator at 25 ± 0.5^0C without controlling light and humidity.

Bishkatali plants were collected from surrounding villages of HSTU campus and dried in the shade. The whole plants were powdered in a grinding machine. Before grinding the plants were well dried in an oven at 40^0C for six hours. The dust was passed through a 60-mesh sieve to obtain fine dust, and was stored in glass jar under laboratory condition. One hundred gram of plant powder was dissolved separately in 300ml of chloroform and ethyl alcohol solvents. The mixtures were stirred for 30 minutes and left to stand for 72 hours and shacked several times at certain intervals. The

mixtures were filtered through Whatman 42 filter paper and left to evaporate the solvent, finally hard brownish extract was obtained. The extracts were preserved in tightly corked bottles and stored in a refrigerator for experimental use.

Mortality test: To test the mortality rate or dose response of *T. castaneum* adult to Bishkatali plant extracts, Residual film method (Busvine, 1971) was applied. The required amount of each extract was dissolved separately in the respective solvent to obtain the concentrations as 500, 250, 125, 62.5 and 31.25mg/ml. Then 1ml solution of each dose (concentration) was dropped onto a petridish (6 cm diameter) with the help of pipette and spread evenly throughout the petridish. The petridishes were then air dried for few minutes. Ten adults (3-6 day old) were released into each petridish. Three replications were made for each dose and control. Control petridishes were treated with the solvent only. The petridishes were then kept in an incubator without food at 25±0.5^{0}C for 72 hrs.

The mortality of beetles was recorded at 24, 48 and 72 hours after treatment. The concentrations were calculated by measuring the dry weight of the crude extract applied into the petridish divided by the surface area of the respective petridish. The mortality (%) was corrected by Abbott's formula (Abbott, 1925) and then subjected to probit analysis according to Finney (1947) and Busvine (1971) using software developed in the Department of Agricultural and Environmental Sciences, University of Newcastle, Upon Tyne, United Kingdom.

Repellency and residual efficacy test

For determining repellency and residual efficacy of Bishkatali extracts against *T. castaneum*, the same doses were used as in mortality test. The repellency test was conducted according to the method of Talukder & Howse (1994). Filter papers (Whatman 40) were cut into two half, and 1ml solution of each dose was applied to each half uniformly with a pipette. The treated half of the papers were then air-dried and attached with the untreated half with a cello-tape at middle in such a way that attachment did not interfere with the free movement of insect from one half to another. Each filter paper was then placed in a petridish (9cm diameter). Twenty adult beetles were released at the centre of each filter paper and a cover was placed on the petridish. For each plant extract and each concentration including controls, three replications were used. The insects present on each half of the paper strip were counted at 2 hours intervals up to 20 hours.

The data were expressed as percentage of repulsion (PR) using the following formula:

PR = (Nc - 50) × 2 Where, Nc = % of insects present in the control half.

Positive values expressed repellency and negative values attractancy. The data (PR) were analyzed using analysis of variance after arcsine transformation. The average values were then categorized according to the following scale (McDonald *et al*. 1970):

Class	Repellency (%)	Class	Repellency (%)
0	> 0.01 to 0.1	III	40.1 to 60
I	0.1 to 20	IV	60.1 to 80
II	20.1 to 40	V	80.1 to 100

To determine the residual effect, ten gram of wheat flour was treated with each concentration of each extract. The treated wheat flour were then air-dried and kept in plastic pots (3.5cm × 4 cm). One pair of newly emerged adult beetle was then released into each pot separately at 7, 12 and 17 days after treatment (DAT) and the mouth of the pot was covered with fine cloth. All treatments and control (only solvent) were replicated three times. The pots were kept in an incubator at 25±0.5^{0}C and 70-75% r.h. The adults from each pot were removed after 7 days of release. The numbers of F_1 adult progeny were counted up from 25 days upto 48 days of insect release.

Inhibition rates (IR) were calculated by the following formula:

IR (%) = C_n - T_n/C_n × 100 Where, C_n = number of insects in control pot.

T_n = number of insect in treated pot.

Data on repellency and residual effect were statistically analyzed using ANOVA and Duncan multiple range test (Duncan, 1951) for comparing means of treatments with control.

Results and Discussion

The mortality (%) of *T. castaneum* adults treated with Bishkatali plant extracts in two solvents and their LD_{50} values are shown in Table 1. The LD_{50} values were 39.26, 22.65, 15.80mg/cm^2 in chloroform extract and in ethyl alcohol extract the values were 35.32, 23.43, 14.85mg/cm^2 after 24, 48, and 72 hours post exposure respectively. Comparing the LD_{50} values, it was observed that the ethyl alcohol extract showed better performance than chloroform extract at 24 and 72 hours whereas the performance of chloroform extract was better at 48 hours. The mortality of beetles ranged from 10-43%, 17-50%, 17-55% in chloroform extract and 7-40%, 10-47% and 17-57% in ethyl alcohol extract at 24, 48 and 72 hours post exposure respectively. The probit regression line of Bishkatali extracts in both solvents indicated that insect mortality positively correlated with the concentrations at all exposure periods (Figure 1).

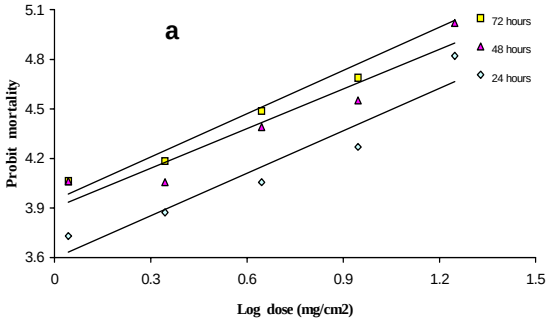
Hussain (1995) observed some toxicity of Bishkatali (*P. hydropiper*) leaf powder and extract on the larvae of *T. castaneum* under laboratory condition. Ferrolino & Padolino (1985) found that the leaf extract of *Artemisia vulgaris* was highly toxic to *T. castaneum* and *Sitophilus zeamis.* Talukder & Howse (1993) have noted toxic effect of Pithraj (*Aphanamixis polystachya*) extract on the red flour beetle. Khanom & Khalequzzaman (2000) investigated the effectiveness of neem seed extracts on larvae and adults of different strains of *T. castaneum* and observed that mortality of the adults varied among different strains. The order of toxicity was found as petroleum spirit >methanol >ethyl acetate >acetone extracts in FSS-II and CTC-12 strains,

while it was found as petroleum spirit >ethyl acetate >methanol >acetone in local and CR-1 strain.

In present study, Bishkatali extracts in both chloroform and ethyl alcohol showed strong repellency against the red flour beetle. Overall, the highest repellency (87.3%) was observed in 500mg/ml extract in chloroform and lowest (52.6%) in 31.25mg/ml extract in ethyl alcohol (Table 2). Depending on the rate of repellency to chloroform extract doses 500, 250 and 125 mg/ml grouped into class V and 62.5 and 31.25mg/ml into class IV. Similar trend of repellency was also observed in ethyl alcohol extract (Table 2). The dose 500 mg/ml was grouped into class V; 250, 125 and 62.5mg/ml into class IV and 31.25mg/ml into class III. The overall results indicated that repellency rate increased proportionally with the increase of concentration of the extract. ANOVA showed that all the doses (concentrations) of both the extracts were differed significantly ($P<0.05$) at different hours of post-treatment (Table 2).

The present result supports the finding of David *et al*. (1988), who showed the repellent activity of *Vitex negundo* against several species of stored products pests. Talukder & Howse (1993) reported strong repellent effect of *A. polystachya* on *T. castaneum*. Hussain *et al.* (1995) also reported that the extract of *P. hydropiper* and *Annona squamosa* had repellent effect against adult *T. castaneum*.

Residual effect of Bishkatali plant extracts in chloroform and ethyl alcohol against *T. castaneum* are



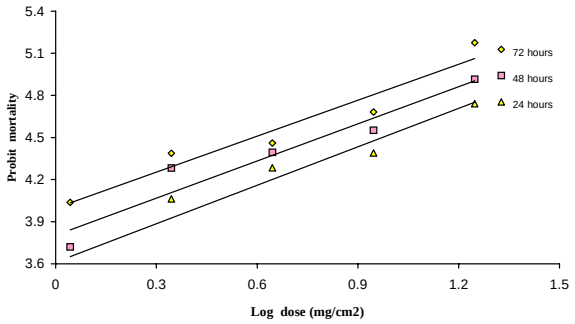


Figure 1 Regression line of probit mortality on log dose of Bishkatali plant extract in chloroform (a) and in ethyl alcohol (b) on adult *T. castaneum* after 24, 48 and 72 hours of application

presented in Table 3. The efficacy of Bishkatali extract as food protectant against the red flour beetle has evaluated by comparing the numbers of F_1 progeny. The lowest number of F_1 adult progeny (32.7, 25.3 and 27.0) has emerged from flour treated with chloroform extract (500mg/10g) when parents released at 7, 12, 17 days after treatment respectively (Table 3). When flour treated with 500mg/10g by ethyl alcohol extract, 38.0, 29.7 and 30.3 F_1 adult progeny emerged when parent released at 7, 12, 17 days after treatment respectively. The results indicated that the Bishkatali extracts in both solvents significantly inhibited the population growth of *T. castaneum*. The highest inhibition was observed as 59.3 and 52.3% in chloroform and ethyl alcohol extracts, respectively at highest dose (500 mg/10g) when adult were released at 12 days after treatment.

Amin *et al.* (2000) found the inhibition activity of Akanda, Bishkatali, Neem extracts against the lesser grain borer. The present results also supports the findings of Pruthi (1937) who reported that neem leaves showed strong protective effect on stored grains from insect infestation. Talukder & Howse (1995) stated that the ground leaves, bark and seeds of *A. polystrachya* provided protection of wheat flour by reducing F_1 progeny of *T. castaneum.* However, the present results revealed that Bishkatali plant extracts in both chloroform and ethyl alcohol had remarkable residual effects on *T. castaneum* by reducing the production of F_1 progeny and/or by increasing the population mortality.

Table 1 Lethal doses (LD_{50}) of Bishkatali plant extract and mortality (%) of *T. castaneum* adults at different exposure periods

Doses (mg/ml)	Extract concentration (mg/cm^2)	Chloroform extract			Ethyl alcohol extract		
		Corrected (%) mortality at			Corrected (%) mortality at		
		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
500	17.68	43	50	55	40	47	57
250	8.84	23	33	38	27	33	37
125	4.42	17	27	31	23	27	30
62.5	2.21	13	17	21	17	23	27
31.25	1.11	10	17	17	7	10	17
LD_{50} values (mg/cm^2)		39.26	22.65	15.80	35.32	23.43	14.85
χ^2 values (df)		0.938(3)	0.882 (3)	0.420 (3)	0.570 (3)	0.715 (3)	0.804 (3)

Note: Control mortality 0.00% at all the exposure periods of both the extracts

Table 2 Repellency effects of Bishkatali plant extracts on *T. castaneum*

Sol-vents	Extract concen-tration (mg/ml)	Mean repellency (%) after treatment at										Overall mean repelle- ncy (%)	Repe-llency class
		2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h		
Chloro-form	500	86.7a	86.7a	86.7a	86.7a	90.0a	86.7a	90.0a	86.7a	86.7a	86.7a	87.3	V
	250	83.3a	86.7a	83.3ac	80.0ab	86.7ab	83.3a	86.7a	83.3a	86.7a	86.7a	84.6	V
	125	76.7ab	76.7ab	76.7ac	76.7bc	80.0bc	80.0ab	83.3ab	83.3a	83.3a	83.3a	80.0	V
	62.5	66.7bc	70.0bc	73.3bc	70.0c	73.3cd	70.0bc	76.7b	70.0b	76.7ab	76.7a	72.3	IV
	31.25	60.0c	60.0c	66.7c	56.7d	66.7d	60.0c	66.7c	63.3b	66.7b	63.3b	63.0	IV
Ethyl alcohol	500	83.3a	83.3a	80.0a	80.0a	80.0a	76.7a	80.0a	80.0a	76.7a	83.3a	80.3	V
	250	73.3ab	73.3b	76.7ab	73.3ab	73.3ab	76.7a	73.3ab	70.0ab	73.3a	73.3ab	73.6	IV
	125	73.3ab	66.7bc	66.7ac	66.7bc	66.7bc	70.0ab	66.7bc	66.7ac	66.7ab	63.3bc	67.3	IV
	62.5	66.7b	60.0cd	63.3bc	60.0cd	60.0cd	63.3bc	60.0cd	60.0bc	56.7bc	56.7c	60.6	IV
	31.25	53.3c	53.3d	53.3c	50.0d	53.3d	53.3c	50.0d	53.3c	53.3c	53.3c	52.6	III

Values within each column of each solvent extract followed by the same letter (s) are not differ significantly at $P < 0.05$ level of probability.

Table 3 Residual effect of Bishkatali plant extracts on F_1 progeny production of *T. castaneum* adults when released at different periods after treatment.

Solvents	Adult released after	Doses (mg/10g)	Mean No. of F_1 progeny	Inhibition rate (IR %)	F-values
Chloroform	7 days	500	32.7 f	45.7	
		250	40.3 e	32.6	
		125	45.7 d	24.2	219.2**
		62.5	48.3 c	19.9	
		31.25	53.0 b	12.1	
		Control	60.3 a	0	
	12 days	500	25.3 f	59.3	
		250	33.7 e	45.9	
		125	40.3 d	35.3	167.5**
		62.5	46.0 c	26.1	
		31.25	54.3 b	12.8	
		Control	62.3 a	0	
	17 days	500	27 .0 f	57.3	
		250	33.3 e	47.3	
		125	41.3 d	37.7	135.7**
		62.5	48.7 c	23.0	
		31.25	55.3 b	12.6	
		Control	63.3 a	0	
Ethyl alcohol	7 days	500	38 .0 f	36.9	
		250	41.7 e	30.8	
		125	46 .0 d	23.7	129.2**
		62.5	49.3 c	18.2	
		31.25	53.0 b	12.1	
		Control	60.3 a	0	
	12 days	500	29.7 f	52.3	
		250	37.7 e	39.4	
		125	41 .0 d	34.1	300.6**
		62.5	47.0 c	24.5	
		31.25	54.3 b	12.8	
		Control	62.3 a	0	
	17 days	500	30.3 f	52.1	
		250	37.3 e	41.0	
		125	41.0 d	34.7	126.3**
		62.5	47.3 c	25.2	
		31.25	55.0 b	13.1	
		Control	63.3 a	0	

Values within column of each solvent and adult release line followed by the same letter(s) are not differ significantly at $P < 0.05$.

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