



TOXICITY AND REPELLENCY EFFECT OF SOME INDIGENOUS PLANT EXTRACTS AGAINST LESSER GRAIN BORER, *RHYZOPERTHA DOMINICA* (F.) (COLEOPTERA: BOSTRYCHIDAE)

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Abstract

Context: Insect bio assay and repellency test can play a vital role in special environmental conditions.

Objectives: To screen out the insecticidal potency of some plant extracts to control the adult lesser grain borer by insect bioassay and repellency tests.

Materials and Methods: Toxicity test of five indigenous plant extracts with three concentrations were conducted against *Rhizopertha dominica*. Insect mortality was recorded at 24, 48, and 72 HAT. For residual toxicity test, insect mortality was recorded at 1, 2, 7, 15 and 21 DAT. The repellent activities were evaluated using the filter paper impregnation method and the data were counted at hourly intervals up to 6th hour. In all cases ten insects per replication were tested and each treatment was replicated thrice. The collected data were statistically analyzed.

Results: Among the tested plant extracts, neem showed the highest toxic and repellent effects against the lesser grain borer. All the doses applied had direct toxicity, residual and repellency effects while 8% dose showed the highest response. The order of toxicity was found as neem > biskatali > karabi > akanda > ata. Mortality percentages were directly proportional to the time after treatment.

Conclusion: This study proved that the leaf extract of indigenous plants like neem, biskatali, karabi ata and akanda can be used to protect stored grain pests.

Key words: Mortality, repellency, residual effect, petroleum ether, *Rhizopertha dominica*.

Introduction

Losses due to insect infestation are the most serious problem in storage, particularly in villages and towns of developing countries like Bangladesh. Storage loss may also be significant in developing countries (70%) (Kavita 2004). It has been estimated that about 15-20% of the world agricultural production is lost every year due to insect infestation (Wright 1985). In Bangladesh, the annual grain losses cost over taka 100 cores (Alam 1971) whereas in India losses caused by insects accounted for 6.5% of stored grain (Kumar 2009). The climate and storage conditions, especially in the tropics, are often highly favorable for insect growth and development (Jacobson 2004). Their attacks reduce both the quantity and quality of stored seed. Sometimes moulds grow in the insect infested food grain and these moulds produce a chemical substance called aflatoxin which is reported to be associated with the liver cancer of human being (Singh 1983). *Rhizopertha dominica* Fab. (Coleoptera: Bostrichidae) is the most common and injurious to stored grains having an important position among the storage pests. It is a field-to-store pest and cause economic damage (Adedire 2001). Both the adults and grubs causes' serious damage to stored grains and stored products and adult beetles are more harmful which destroy healthy grains and reduced them to frass. They destroy far more than they consume.

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Synthetic chemical pesticides have been used for many years to control stored grain pests (Salem *et al.* 2007). Fumigation of stored food grains with toxic gases is effective but not applicable at the farm level because the storage structures are not airtight. Furthermore, control of insects by insecticides has serious drawbacks, such as the toxic residues on stored grains, development of resistance by target species, pest resurgence and lethal effects on non-target organisms in addition to direct toxicity to users and health hazard (Adedire and Lajide 2003, Adedire *et al.* 2011, Ileke and Oni 2011, Udo, 2011, Ileke and Olotuah 2012, Ileke and Bulus 2012). This situation indicates the need for safe but effective, biodegradable pesticides with no toxic effects on non-target organisms for pest control in storage. Recently, there is a steady increase in the use of indigenous plant products as a cheaper and ecologically safer means of protecting stored products against infestation by insects (Ashamo and Odeyemi 2001, Oni and Ileke 2008, Akinkulere *et al.* 2009, Ileke *et al.* 2012) and this lead to the present study.

Materials and Methods

Preparation of plant extracts

Fresh leaves of ata (*Annona reticulata* L.), karabi (*Nerium oleander* L.), neem (*Azadirachta indica* L.), biskatali (*Polygonum hydropiper* L.) and akanda (*Calotropis gigantea* L.) leaves extracts were used against *R. dominica* in the laboratory, Entomology Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur during April to December 2012. They were collected around the HSTU campus. They were washed in running water and kept in laboratory for 7 days air drying. After drying they were made powder separately by an electric grinder. The extracts were prepared according to (Chitra *et al.* 1993) with minor modifications. For making extracts, 100 g of different plant powders were dissolved in 300 ml of petroleum ether solvent and stirred for 30 min. in a magnetic stirrer. The mixture was allowed to stand for 72 hours and shaking several intervals. It was filtered through a filter paper (Whatman no. 1) and to evaporate the solvents. The condensed extracts were preserved in tightly corked-labeled bottles and stored in a refrigerator until their further use.

Collection of wheat grains

Healthy wheat grains, *Triticum aestivum* (L.) were purchased from the local market of Dinajpur town, cleaned thoroughly and sun dried. The grains were cooled at 8-10% moisture level and stored at room temperature in air tight plastic bag for experimental use.

Mass rearing of *Rhyzopertha dominica*

R. dominica were collected from the naturally infested wheat grains from the local market of Dinajpur and was mass reared in the laboratory at ambient room temperature ($28\pm 0.5^{\circ}\text{C}$) in glass jars (47 cm height \times 4 cm dia). Approximately 200 adults were released in each glass jar containing 500 g of wheat grains and the mouth was closed with a piece of cloth fastened with rubber band to prevent contamination and escape of insect. After oviposition, the adults were separated from the grains by sieving and seeds along with eggs were left in the container for emergence of next generation. The newly emerged adults (1-7- days- old) were collected and again allowed for oviposition with new grains in different containers to maintain a stock culture of the test insect. The process was continuing for getting enough pest throughout the study.

Evaluation of toxicity of different plant extracts

Toxicity test were conducted according to (Talukdar and Howse 1993) with minor modifications. The extracted materials were weighed and dissolved in petroleum for making different concentration (4.0, 6.0 and 8.0 % along with control). Pilot experiments were done to obtain the appropriate dose. Before applying extracts to the thorax of the insect, 10 minutes chilling were done with 4°C in refrigerator. Then $1\ \mu\text{l}$ of prepared solution was applied to the dorsal surface of each insect using a micropipette (volume digital micropipets, bio-rad, India). Ten insects per replication were treated and each treatment was replicated

thrice. In addition, the same numbers of insects with petroleum ether solvent only were treated as control. After treatment, the insects were transferred into petridishes (9 cm diameter). Mortality was recorded after 24, 48, and 72 hours treatment (HAT) (Talukdar and Howse 1993). The data were corrected according to Abott's (1987) formula.

Determination of residual effect

Three different concentrations of each plant-extract were prepared with the petroleum ether solvent. Then 1 ml of prepared solution was applied to 50g wheat and mixed properly. After 10 minutes air-dried five pairs (1-day-old) insects were released into the pot containing plant extracts treated wheat grain and then pot was covered with perforated lid. Three replications were maintained for each of the concentration of the individual plant extracts along with control. All treated pots were kept at ambient temperature ($28 \pm 0.5^\circ\text{C}$) in the laboratory. Mortality was recorded at 1, 2, 7, 15 and 21 DAT (days after treatment).

Detection of repellency

First of all, the Whatman No. 1 filter papers were cut into two half. With the help of a pipette 1 ml solution of each plant extract was applied to one half of the filter paper and only petroleum ether solvent was used for another half as control. The treated half and control half was then air-dried. Ten insects were released at the centre of each petridish with a cover. For each plant extract and each dose three replications were used. The insect present on each portion were counted at hourly intervals up to 6th hour. The data were expressed as percentage repulsion (%PR) by the following formula (Talukdar and Howse 1994):

$$\% \text{ PR} = (N_c - 50) \times 2$$

Where, % PR = percentage repulsion, N_c = percentage of insects present in the control half.

Positive (+) values expressed repellency and negative (-) values attractency. The average values were categorized according to the following scale (McDonald *et al.* 1970)

Class		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

Statistical analysis

The collected data were statistically analyzed by completely randomized design (CRD) using MSTAT statistical software. The treatment mean values were adjusted by Duncan's New Multiple Range Test (DMRT) and mortality data subjected to probit analysis.

Results and Discussion

Effects of direct toxicity against lesser grain borer

Mortality was differed significantly ($p < 0.001$) among all the concentration level at different time interval of different plant extracts. All plant extracts except Kkarabi achieved hundred percent mortality at 72 HAT in 8.0% concentrations. Average mortality percentage of indicated that neem leaf extract (98.90 %) possessed the highest toxic effect followed by biskatali (94.43%) and karabi (56.63%) possessed the lowest toxic effect (Table 1). The order of toxicity of five plant extracts were found as neem > biskatali > akanda > ata > karabi.

The above findings revealed that all tested plants extracts are toxic against lesser grain borer and the neem plant extracts showed the highest toxic effect. This finding agreed with Ileke and Bulus (2012) who work with the response of *R. dominica* to powders and extracts of *Azadirachta indica* and *Piper guineense* seeds and showed that adult mortality increased both concentration of powders and extracts.

Table 1. Interaction effects of plant extract and concentration on lesser grain borer at different HAT (Hours after treatment).

Plant extracts used	Concentrations (%)	% Insect mortality (HAT)			
		24	48	72	Average
Neem	0.0	0.00h	0.00g	0.00i	0.00
	4.0	40.00d	76.67bc	80.00bc	65.57
	6.0	56.67c	90.00ab	100.0a	82.23
	8.0	96.67a	100.0a	100.0a	98.90
Biskatali	0.0	0.00h	0.00g	0.00i	0.00
	4.0	16.67fg	23.33f	40.00ef	26.67
	6.0	40.00d	76.67bc	90.00ab	68.90
	8.0	83.33b	100.0a	100.0a	94.43
Karabi	0.0	0.00h	0.00g	0.00i	0.00
	4.0	3.33gh	3.33g	20.00h	8.90
	6.0	16.67fg	36.67ef	46.67de	33.37
	8.0	33.33de	60.00d	76.67c	56.63
Akanda	0.0	0.00h	0.00g	0.00i	0.00
	4.0	23.33ef	23.33f	26.67gh	24.43
	6.0	36.67de	63.33cd	73.33c	57.77
	8.0	76.67b	96.67a	100.0a	91.10
Ata	0.0	0.00h	0.00g	0.00i	0.00
	4.0	16.67fg	23.33f	33.33fg	24.43
	6.0	33.33de	43.33e	56.67d	44.47
	8.0	60.00c	86.67ab	100.0a	82.20
P- value		0.0001	0.0001	0.0001	-
LSD		13.24	14.98	11.98	-
CV (%)		4.29	4.06	3.90	-

HAT = Hours after treatment, Mean followed by column the same letter(s) did not differ significantly at 5% level by DMRT.

Probit analysis for direct toxicity

The LD₅₀ values of neem (6.958 µg), biskatali (8.444 µg), karabi (9.191 µg), akanda (10.589 µg) and ata (14.522 µg) at 24 HAT (Table 2) indicated that neem leaf extract was found to be highly toxic. Similarly, neem leaf extract maintain its highest toxicity at 48 and 72 HAT. The results obtained in this probit study showed that all the tested plants would be more or less effective for controlling lesser grain borer but neem was most effective. The lowest LD₅₀ values of neem plant extract indicated that the highest toxic effects against lesser grain borer to suppress their population growth in treated wheat grains. Some researchers who had earlier evaluated *A. indica* powder and extract as botanical insecticides and grains protectant had found them to be effective against *S. zeamais* and *C. maculatus* (Onu and Baba 2003, lleke and Oni 2011, lleke and Bulus 2012). The toxicity of neem to stored products insects has been attributed by various authors to the presence of many chemical ingredients such as triterpenoids, which includes azadirachtin, salanin, meliantriol (Mbailao *et al.* 2006, lleke and Oni 2011).

Effect of residual toxicity against lesser grain borer

The effects of residual toxic of plant extracts, doses and time revealed that neem plant extract possessed the highest residual effect (average mortality, 98.97%) followed by biskatali (average mortality 95.20 %) against lesser grain borer at maximum dose (8.0 %). Mortality percentages were differed significantly between plant extracts and doses (Table 3). The toxic effect of five plant extracts was: neem > biskatali > karabi > akanda > ata.

Table 2. Relative toxicity (by probit analysis) of different plant extracts treated against lesser grain borer at 24, 48 and 72 HAT.

Name of the plant extracts	No. of insect used	LD 50 values (μg)	95 % fiducially limits	χ^2 values
24 HAT				
Neem	90	6.958	3.646 - 13.278	0.0055
Biskatali	90	8.444	4.251 - 16.770	0.0514
Karabi	90	9.191	4.262 - 19.822	0.0943
Akanda	90	10.589	4.827 - 23.226	0.1735
Ata	90	14.522	3.644 - 57.870	0.3752
48 HAT				
Neem	90	1.448	0.021 - 97.257	0.0439
Biskatali	90	3.709	1.716 - 8.013	0.0015
Karabi	90	5.771	4.4933 - 7.412	0.2081
Akanda	90	6.349	4.947 - 8.148	0.0476E-04
Ata	90	9.342	5.466 - 15.966	0.1611
72 HAT				
Neem	90	2.744	1.630 - 4.755	0.3102
Biskatali	90	2.784	1.449 - 5.195	0.0730
Karabi	90	3.432	2.131 - 5.528	0.0339
Akanda	90	4.127	2.940 - 5.794	0.1356
Ata	90	5.924	4.989 - 7.034	0.0116

HAT= Hour after treatment, Values were based on three concentrations, three replications of 10 insects each, χ^2 = Goodness of fit, the tabulated value of χ^2 is 5.99 (d. f=2 at 5% level).

Table 3. Interaction effects of plant extract and concentration on the lesser grain borer at different DAT (Days after treatment).

Plant extracts used	Concentrations (%)	% Insect mortality (DAT)					Average
		1 DAT	2 DAT	7 DAT	15 DAT	21 DAT	
Neem	0.0	0.00h	0.00f	0.00g	0.00i	0.00i	0.00
	4.0	40.00d	56.67d	76.67bc	78.33bc	80.00bc	66.33
	6.0	55.67c	75.33c	90.00ab	96.00ab	100.0a	83.40
	8.0	94.33a	95.67a	100.0a	100.0a	100.0a	98.97
Biskatali	0.0	0.00h	0.00f	0.00g	0.00f	0.00i	0.00
	4.0	15.67fg	20.33e	23.33f	29.67g	55.00ef	28.80
	6.0	38.00d	46.33c	76.67bc	85.67a	93.00ab	67.93
	8.0	83.33b	92.67b	100.0a	100.0a	100.0a	95.20
Karabi	0.0	0.00h	0.00f	0.00g	0.00f	0.00i	0.00
	4.0	20.33ef	22.33e	23.33f	25.33ef	36.67gh	25.60
	6.0	35.33de	46.67cd	63.33cd	69.67c	79.33c	58.87
	8.0	74.67b	88.67b	96.67a	100.0a	100.0a	92.00
Akanda	0.0	0.00h	0.00f	0.00g	0.00f	0.00i	0.00
	4.0	13.67fg	21.67e	22.33f	34.67e	35.33fg	25.53
	6.0	30.33de	40.67c	48.33e	54.67c	59.67d	46.73
	8.0	58.33c	76.67b	89.67ab	95.33bc	100.0a	84.00
Ata	0.0	0.00h	0.00f	0.00g	0.00f	0.00i	0.00
	4.0	7.33gh	9.33f	12.33g	16.67f	20.00h	13.13
	6.0	16.33fg	25.67de	39.67ef	45.33de	49.67de	35.33
	8.0	35.67de	49.67c	66.00d	70.00c	80.67c	60.40
P- value	0.0001	0.0001	0.0001	0.0001	0.0001	-	
LSD	14.24	14.98	15.98	13.98	12.98	-	
CV (%)	5.29	6.20	7.06	4.65	3.90	-	

DAT = Days after treatment, Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT.

Probit analysis for residual toxicity

The LD₅₀ values of neem (18.543 µg), biskatali (29.817 µg), karabi (30.937 µg), akanda (34.883 µg) and ata (31.204 µg) at 1 DAT (Table 4) indicated that neem plant extracts possessed the highest toxicity while lowest in akanda against the test insect. Similarly, neem plant extract maintained its highest toxicity at 2, 7, 15 and 21 DAT.

The chi-square (χ^2) values of different plant extracts at different hours after treatment were insignificant. It is clear that neem leaf extracts possessed the highest toxicity in controlling lesser grain borer. The compound azadirachtin may work as an Insect Growth Regulator (IGRs) interfering with ecdysone which prevents immature insects from molting (Soon and Bottrell 1994).

Table 4. Relative toxicity (by probit analysis) of different plant extracts treated against lesser grain borer at 1, 2, 7, 15 and 21 DAT.

Name of the plant extracts	No. of insect used	LC 50 values (µg)	95 % fiducially limits	χ^2 values
1 DAT				
Neem	90	18.543	2.721 – 126.347	0.019
Biskatali	90	29.817	1.071 – 830.053	0.051
Karabi	90	30.937	1.388 – 689.277	0.095
Akanda	90	34.883	1.432 – 849.918	0.023
Ata	90	31.204	1.648 – 590.492	0.082
2 DAT				
Neem	90	11.446	3.591 – 36.476	0.097
Biskatali	90	12.573	3.974 – 39.774	0.241
Karabi	90	19.754	2.579 – 151.281	0.214
Akanda	90	31.204	1.648 – 590.492	0.082
Ata	90	15.259	4.986 – 246.693	0.052
7 DAT				
Neem	90	5.941	4.833 – 7.302	0.378
Biskatali	90	6.312	5.131 – 7.765	0.405
Karabi	90	8.267	5.983 – 11.421	0.103
Akanda	90	11.057	7.065 – 17.303	0.238
Ata	90	10.243	6.164 – 17.023	0.339
15 DAT				
Neem	90	4.431	3.580 – 5.482	0.240
Biskatali	90	4.508	3.500 – 5.803	0.139
Karabi	90	5.412	4.609 – 6.354	0.559
Akanda	90	8.224	6.663 – 10.149	0.001
Ata	90	6.240	5.325 – 7.312	0.002
21 DAT				
Neem	90	3.765	2.829 – 5.010	0.341
Biskatali	90	3.803	2.849 – 5.076	0.217
Karabi	90	5.259	3.268 – 5.549	0.629
Akanda	90	6.429	5.381 – 7.679	0.973
Ata	90	4.849	3.879 – 6.062	0.005

DAT= Day after treatment, Values were based on three concentrations, three replications of 10 insects each, χ^2 = Goodness of fit, the tabulated value of χ^2 is 5.99 (d. f=2 at 5% level).

Effect of repellency on lesser grain borer

Mean repellency effect of different plant extracts in different dose level on lesser grain borer is presented in Table 5. The repellency was influenced by the concentration of extracts. The rate of the extract and the repellency class were found I to V in all five plant extracts.

Table 5. Repellency effect of different plant extract and their doses on lesser grain borer at different HATs.

Plant extracts used	Repellency rate (%) at six different HATs								
	Dose (%)	1 st hour	2 nd hour	3 rd hour	4 th hour	5 th hour	6 th hour	Average	Repel. class
Neem	0.0	0.00f	0.00e	20.00de	0.00c	0.00f	0.00g	3.30	I
	4	33.33a-f	26.67b-e	20.00de	26.67bc	26.67c-f	26.67d-g	26.67	ϣ
	6	53.33a-d	26.67b-e	33.33c-e	20.00c	33.33b-f	13.33fg	30.03	ϣ
	8	66.67ab	53.33ab	100.0a	93.33a	86.67a	93.33a	82.20	V
Biskatali	0.0	0.00f	20.00b-e	20.00de	0.00c	0.00f	0.00g	6.70	I
	4	26.67b-f	40.00a-d	40.00b-e	26.67bc	53.33a-d	66.67a-d	42.20	ω
	6	60.00a-c	40.00a-d	46.67b-d	73.33ab	80.00a	80.00ab	63.33	IV
	8	53.33a-d	26.67b-e	13.33de	46.67bc	20.00d-f	33.33c-g	32.23	ϣ
Karabi	0.0	0.00f	0.00e	0.00e	40.00bc	0.00f	0.00g	6.70	I
	4	26.67b-f	66.67a	73.33a-c	46.67bc	60.00a-c	66.67a-d	56.67	ω
	6	6.66ef	60.00a	46.67bcd	40.00bc	13.33ef	20.00e-g	31.13	ϣ
	8	40.00a-f	46.67a-c	26.67de	26.67bc	60.00a-c	53.33a-f	42.20	ω
Akanda	0.0	0.00f	0.00e	80.00ab	0.00c	0.00f	20.00efg	16.70	I
	4	46.67a-e	66.67a	46.67b-d	46.67bc	66.67ab	33.33c-g	51.13	ω
	6	73.33a	33.33a-e	40.00b-e	40.00bc	13.33ef	60.00a-e	43.33	ω
	8	33.33a-f	60.00a	33.33c-e	33.33bc	40.00b-e	40.00b-g	40.00	ω
Ata	0.0	20.00c-f	0.00e	0.00e	20.00c	0.00f	0.00g	6.700	I
	4	13.33d-f	20.00b-e	13.33de	33.33bc	20.00d-f	33.33c-g	22.23	ϣ
	6	20.00c-f	6.66de	20.00de	6.66c	40.00b-e	66.67a-d	26.67	ϣ
	8	46.67a-e	13.33c-e	20.00de	33.33bc	20.00d-f	73.33a-c	34.43	ϣ
P- value	0.0867	0.0439	0.0002	0.0071	0.0002	0.0021	-		
Lsd	36.33	28.70	36.95	39.76	33.25	38.76	-		
CV (%)	4.91	3.23	4.48	3.63	3.52	3.12	-		
SE	12.69	10.02	12.91	13.89	11.61	13.54	-		

Mean followed by the same letter(s) did not differ significantly at 5% level by DMRT.

The highest mean repellency (82.20%) was found in neem extract at 8% dose whereas lowest (3.30%) in control treatment. With the progress of time, the repellency effect decreased in maximum cases. Neem products repel insects, stop their feeding, inhibit reproduction and cause other interruptions (Schmutterer 1990). Jilani and Saxena (1990) observed that the repellency of compounds with low molecular weights and high volatility decreased rapidly over time.

Conclusion

The present study revealed that the highest mean repellency was observed in neem extract for lesser grain borer, *R. dominica*. This study is proved our traditional use of leaves of neem, biskatali, karabi ata and akanda to protect stored grain pests.

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