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Evaluation of some indigenous plant extracts against red flour beetle

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Abstract: An investigation was carried out in the laboratory with acetone and water extracts of eucalyptus (*Eucalyptus globules),* Nishinda*,(Vitex negundo),* Datura*, (Datura fastuosa)* and Pithraj, (*Aphanamixis polystachya)* at 100, 75 50 and 25 mg/ml concentrations to evaluated for their toxic effects against the red flour beetle, *Tribolium castaneum* The results showed that extracts of all the four plants had toxic on the insect. The Nishinda leaf extract was the most toxic for red flour beetle (mortality,42.12%). The results also showed that actetone extract was more effective than water extract. The effectiveness of the extracts of the test plants increased with the increase of concentration.
Key words: Evaluation, plant extract, Red flour beetle.

Introduction

Insect infestation on stored grains and their products is a serious problem throughout the world. Infestation of insects occurs in various bulk stored commodities and processed plants and causes economic damage to raw goods and finished products (Irshad and Jilani, 1990; Arther and Zettler, 1991). There are approximately 200 species of insects and mites attacking stored grains and stored products (Maniruzzaman,1981).

Red flour beetle is a serious pest and occurs widely throughout the world (Anon,1973). Both grubs and adults of red flour beetle feed on a wide range of commodities and is an important pest of stored cereal (Husain, 1995).

To minimize the loss of stored grains due to insect attack, Bangladesh has to import a large amount of chemical insecticides by spending a huge amount of foreign exchange every year. Synthetic insecticides which have been in use for a long time for controlling insect pests, have got many limitations and undesirable side effects (Ahmed *et al*., 1981; Khanam *et al.,* 1990; Haque and Husain, 1993).

Botanical plant products are environmentally safe, less hazardous and less expensive. The main advantage of botanical insecticides is that they can be easily produced by the farmers in the house and small-scale industries. The farmers of India were reported to save their crop and/or products with herbal substances such as oils, leaves, roots, seeds etc. of different plant instead of synthetic chemical insecticides for a long time (Talukder and Howse, 1993).

However, a very few scientific research works have been done in Bangladesh to explore our locally available plant materials for the control of harmful insect pests in storage and field level. These botanical materials can be used as an alternative to chemical pesticides. This will be very helpful in minimizing the undesirable side effects of synthetic pesticides. The present study was, therefore, undertaken to evaluate the toxic effects of plant extract of Eucalyptus, Nishinda, Datura and Pitraj in controlling red flour beetle.

Materials and Methods

The experiment was conducted in the laboratory of the Department of Entomology, Bangladesh Agricultural University (BAU), Mymensingh and Division of Entomology, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, during the period from May 2004 to February 2005. Fresh plant materials i.e. leaves of Eucaluptus(*Eucalyptus globules*), Nishinda (*Vitex negundo* L.), Datura (*Datura fastuosa* L) and Pithraj (*Aphanamixis polystachya*) were collected from different areas of Mymensingh. The collected leaves were washed and air dried then in the oven. Dried leaves were then powered in an electric grinder.

Collection and Rearing of Insect: Red flour beetles were collected from the Division of Entomology, BINA, Mymensingh. Then they were maintained in the growth chamber at 27-30^{0}C and 70-75%RH. The insects were reared on sterilized wheat grains in rectangular jars of size14x10x30cm. Each jar was set up with 80 pairs of adults beetles. Wheat grains and wheat flour were sterilized at 60^{0}C for 30 minutes. Then they were used as food for red flour beetle. The jares were covered with a piece of cloth fastened with rubber band to prevent contaminations and insect escape. After allowing them for free ovipositors for a period of 7 days, all the adults of red flour beetle were removed from each jar. Then the jars were put back in to growth chamber for completing the generation of insects.

Preparation of plant dust: The collected dried leaves were ground to powder in an electric grinder. A 25 mesh diameter sieve was used to obtain fine dust.

Methods of Extraction: Previously prepared leaf dusts were used for preparation of plant extract. Ten grams of each category dust were taken in a 600ml beaker and separately mixed with 100 ml different solvents (acetone, and distilled water). Then the mixture was stirred for 30 minutes by a magnetic stirrer stirrer (at 6000rpm) and left to stand for next 24 hours. The mixture was then filtered through a fine cloth and again through filter paper (Whatman No-1.). The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvents in a water bath at 45^{0}C, 55^{0}C and 80^{0}C temperature for acetone and water extracts respectively. After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labeled bottles and stored in a refrigerator until their use for insect bioassays.

Preparation of stock solution: 100, 75, 50 and 25 mg/ml solutions of each category of plant extracts were prepared dissolving the extract in the respective solvent prior to insect bioassay.

Disinfestations of wheat grains: The wheat grains were spread on the black polythene sheet and sun dried from 9 a.m.to 2 p.m.in sun-light with air temperature ranging from 32-42^{0}C for five consecutive days. Sun-dried grains were kept in the oven at 45^{0}C for few hours, packed in polythene bag and sealed to avoid future infestation.

Insect Bioassays: The present study was designed with following types of bioassays to determine the direct toxicity, residual and repellent effects against *T.castaneum.*

Toxicity tests (by topical application method): A laboratory test for direct toxicity by topical method was conducted according to the method of Talukder and Howse (1993). Four different concentrations of each plant extracts (100, 75, 50 and 25mg/ml) were prepared with respective solvents. Then one ul of prepared solution was applied to the dorsal surface of the thorax of each insect using a micro-capillary tube. Ten insects per replication were treated and each treatment was replicated 3 times. In addition, the same numbers of insects were treated with solvent only for control. After treatment, the insects were transferred into 9 cm diameter Petridishes (10 insects/petridish) containing food (wheat grains). Insect mortalities were recorded by at, 48 and 72 hours after treatment (HAT). Original data were corrected by Abbott (s) (1987) formula and then transformed into arcsine percentages before ANOVA.

$$P = \frac{P - C}{100 - C} \times 100$$

Where, P=Percentage of corrected mortality, p= Observed mortality (%), C= Control mortality (%). The Probit analyses were done to estimate LC_{50} values with their 95% fiducial limits and the slopes of regression lines were also calculated.

Statistical Analysis: All the experimental data were statistically analyzed by Completely Randomized Design (factorial CRD) using MSTAT statistical software in a microcomputer. The mean values were adjusted by Duncan`s Multiple Range Test (DMRT) (Duncan, 1951).

Results and Discussion

Toxic Effect: The result of the direct toxic effects of different extracts of leaves of Eucalyptus, Nishinda, Datura and Pitraj against red flour beetle, *T castaneum* are presented in Table 1-5. The concentrations used were 100, 75, 50 and 25 mg/ml.)

The order of toxicity of the plant extracts on red flour, *T castaneum* was: Nishinda > Eucalyptus > Datura > Pithraj. It was observed that the toxicity of different plant extracts was influenced by the solvents and time 1. Average value indicated that the highest mortality of red flour beetle. *T castaneum* was found in the acetone extract of Nishinda (43.95%) and the lowest was found in the water extract of Pithraj (22.67%).

The interaction effects of plant, concentration and time is presented in Table 2. The effects of plant, concentration and time had no significant effect on insect mortality on red flour beetle, *T castaneum* except their average values. Here the highest avaraage mortality (57.95%) was found from the extracts on Nishinda plant at 100mg/ml and lowest from the extracts of Pithraj at 25mg/ml concentration level.

Table 1. Toxic effect of different plant extracts of different solvents on red flour beetle, *T castaneum* at different HAT (Interaction of plant, solvent and time)

Name of the plant	Name of the solvents used in extracts	Mortality percentage			
		24 HAT	48 HAT	72 HAT	Average
Eucalyptus	Acetone	28.39	37.97	49.23	38.53 c
	Water	27.03	34.25	44.62	35.30 d
Nishinda	Acetone	33.92	42.39	55.56	43.95 a
	Water	31.91	38.43	50.53	40.29 b
Datura	Acetone	21.41	30.72	40.81	30.98 e
	Water	20.61	28.21	35.22	28.01 f
Pithraj	Acetone	15.97	25.73	33.83	25.18 g
	Water	15.09	23.80	29.11	22.67 h

HAT=Hours after treatment, NS=Not significant; Within column values followed by different letter(s) are significantly different at 0.05 level by DMRT.

Probit Analysis for Direct Toxic Effect: The result of the probit analysis for the estimation of LC 50 values and their 95% fiducial limits and the slope of regression line at 24.48 and 72 HAT for the mortality of red flour beetle are presented in Table (3-5).

Among the solvents used for preparation of extract, the LC50 values indicated that acetone extract of Nishinda leaf was the most toxic (14.78%) followed by water extract of Nishinda leaf (15.98%) at 24 HAT (Table 3). LC50 values at 48 HAT showed that water extract of Nishinda leaf was the most toxic (10.43%) followed by Acetone extract of Nishinda (10.64%) (Table 4). The LC50 values 72 HAT showed that acetone extract of Nishinda leaf was the highest toxic (7.14%) and it was followed by water extract of Nishinda (7.88%).(Table 5).

From the probit results, it is clear that Nishinda will be more effective for controlling the red flour beetle. The chi-square (χ^2) values of different plant extracts at different hours after treatment were insignificant at 5% level of probability except values with star marks (*). Insignificant χ^2 value did not show any heterogeneity of the mortality data.

Probit Regression Lines: The probit regression lines for the effect of four different plant extracts (Eucaluptus, Nishinda, Datura and Pithraj) were calculated.

The probit regression lines for the effects of four different plant extracts on red flour beetle showed a clear linear relationship between probit mortality and their log concentrations. The probit regression lines become steeper as concentrations increased, because the adult insects were treated with more toxics for the same period at higher concentrations.

Table 2. Mean mortality of red flour beetle by different plant extract at different concentration by stomach poisoning

Name of the plant	Concentration (mg/ml)	Mortality percentage		
		24 HAT	48 HAT	72 HAT
Eucalyptus	100	42.12	50.08	63.69
	75	29.88	39.35	54.60
	50	22.77	32.55	38.27
	25	16.06	22.44	31.13
Nishinda	100	45.65	57.21	70.99
	75	38.59	44.39	61.85
	50	28.07	35.76	45.52
	25	19.35	24.27	33.79
Datura	100	29.89	42.89	52.78
	75	24.59	32.33	40.10
	50	17.06	23.45	32.96
	25	12.50	19.19	26.20
Pithraj	100	20.53	35.76	45.52
	75	16.56	25.03	32.79
	50	14.37	21.44	27.36
	25	10.65	16.81	20.22

HAT=Hours after treatment; NS=Not significant

Table 3. Relative toxicity (by probit analysis) of different plant extracts of different solvents treated against on red flour beetle, *T casaneum* at 24 HAT

Name of the plants	Name of the solvents	No of insects used	LC50 values (%)	95%fiducial limits	χ^2 values	Slope±SE
Eucalyptus	Aceton	120	18.58	12.43-27.78	8.03*	2.05±0.04
	water	120	22.59	18.90-27.02	5.83	1.60±0.03
Nishinda	Aceton	120	14.78	10.85-20.15	1034*	2.03±0.03
	water	120	15.98	11.39-22.44	6.59*	2.04±0.04
Datura	Aceton	120	55.21	36.42-83.68	1.37	1.22±0.04
	water	120	49.27	33.75-71.93	0.70	1.28±0.04
Pithraj	Aceton	120	65.79	54.69-77.97	0.65	1.07±0.04
	water	120	75.47	62.11-91.68	1.00	0.95±0.04

HAT=Hours after treatment; Values were based on four concentrations, three replications of 10 insets each χ^2 =Goodness of fit; The tabulated value of X^2 value X^2 is 5.99(d.f.=2 at 5% level)

Table 4. Relative toxicity (by probit analysis) of different plant extracts of different solvents treated against on red flour beetle, *T casaneum* at 48 HAT

Name of the plants	Name of the solvents	No of insects used	LC50 values (%)	95% fiducial limits	χ^2 values	Slope±SE
Eucalyptus	Aceton	120	12.01	9.81-14.69	4.15	2.55±0.04
	water	120	11.52	9.14-14.52	4.18	2.03±0.03
Nishinda	Aceton	120	10.64	8.79-12.87	1.87	2.28±0.03
	water	120	10.43	8.47-12.83	0.86	2.02±0.03
Datura	Aceton	120	27.19	22.01-33.58	2.83	1.62±0.04
	water	120	32.61	24.71-43.05	1.71	1.28±0.03
Pithraj	Aceton	120	45.45	31.94-67.54	7.45*	1.18±0.03
	water	120	93.31	75.15-115.87	3.08	0.81±0.03

HAT=Hours after treatment; Values were based on four concentrations, three replications of 10 insets each χ^2 =Goodness of fit ; The tabulated value of X^2 value X^2 is 5.99(d.f.=2 at 5% level)

Table 5. Relative toxicity (by probit analysis) of different plant extracts of different solvents treated against on red flour beetle, *T casaneum* at 72 HAT

Name of the plants	Name of the solvents	No of insects used	LC50 values (%)	95%fiducial limits	χ^2 values	Slope±SE
Eucalyptus	Aceton	120	8.57	5.85-12.57	0.62	2.84±0.03
	water	120	8.24	5.29-12.83	0.36	2.33±0.03
Nishinda	Aceton	120	7.14	6.14-8.30	4.09	1.93±0.03
	water	120	7.88	6.71-9.25	3.11	1.95±0.03
Datura	Aceton	120	16.47	14.01-19.36	6.29*	1.24±0.03
	water	120	20.28	17.02-24.17	2.57	1.42±0.03
Pithraj	Aceton	120	18.12	15.27-21.47	4.94	1.29±0.03
	water	120	28.74	21.02-39.29	4.17	0.95±0.03

HAT=Hours after treatment; Values were based on four concentrations, three replications of 10 insets each χ^2 =Goodness of fit; The tabulated value of X^2 value X^2 is 5.99(d.f.=2 at 5% level)

The calculated probit regression equations of different plant extracts at 24 HAT were:Y=1.2431x + 2.2148, Y=1.2721x + 2.3194, Y=1.0371x + 2.3615 and Y=0.6661x + 2.8161x for Eucalyptus, Nishinda, Datura and Pithraj respectively (Figure). The regression line for Nishinda olant extract showed the highest and Pithraj showed the lowest probit mortality.

The calculated probit regression equations of different plant extracts at 48 HAT were: Y=1.216x + 2.5021, Y=1.39998x + 2.304, Y= 1.1039x + 2.5175, Y=0.9084x + 2.721 for Eucalyptus, Nishinda, Datura and Pithraj respectively (Figure 4.01). The regression line for Nishinda plant extract showed the highest and Pithraj showed the lowest probit mortality.

The calculated probit regression equations of different plant extracts at 72 HAT were: Y=1.414x + 2.4543, + Y=1.6234x + 2.2492, Y=1.1098x + 2.7448, Y=1.1052x + 2.5754 for Eucalyptus, Nishinda, Datura and Pithraj respectively.

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