SUITABILITY OF SOME BOTANICAL PESTICIDES (NEEM, GARLIC AND RED CHILI) AGAINST DRY FISH INSECTS (Dermestes sp. LARVAE AND Necrobia sp. ADULT)

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ABSTRACT

Acetone extracts of red chili (*Capsicum frutescens*), garlic (*Allium sativum*) and neem (*Azadirachta indica*) were used to evaluate their insecticidal effects against a dry fish beetle, *Dermestes sp.* larvae and a mite, *Necrobia sp.* adult. The concentration of plant extracts were 60, 80 and 100% for *Dermestes sp.* larvae and 10, 15 and 20% for *Necrobia sp.* adult. The mortality of *Necrobia sp.* at 24, 48 and 72 hours after treatment (HAT) indicated that red chili extract possessed the highest insecticidal effect followed by neem and garlic. Mortality was found to be directly proportional to the concentration of plant extracts. For *Necrobia sp.* (adult) LC₅₀ values of red chili, neem and garlic were 0.78, 0.85 and 1.68%, respectively at 24 HAT. Red chili was found to possess the highest insecticidal effect among these three with the highest toxic effect at 48 HAT (0.29%) and 72 HAT (0.03%). For the larvae of *Dermestes sp.*, the LC₅₀ values of red chili was 0.60%, neem 0.69% and garlic 1.15% at 24 HAT, again indicating that red chili had the highest insecticidal effect on dry fish insects.

Key Words: Neem, garlic, red chili, toxic effects, dry fish

INTRODUCTION

In Bangladesh, during storage dry fishes are attacked by several species of dermestid beetle, viz., *Dermestes maculatus*, *D. frischii*, *D. ater* and mite, *Necrobia rufipes* (FAO, 1981; Nowsad, 1995). Fish that are cured and has low moisture content provides a food for beetles, particularly the larvae and to a lesser extent, the adults of *Dermestes sp.* and *Necrobia sp.* The damaged caused by insect infestation is an important cause of economic and physical loss of dry fish in tropical countries (Poulter et al. 1988). Losses of cured fish can be as much as 40% during storage and losses of up to 30% have been caused by beetle and mite infestation (FAO, 1981; Meynell, 1978).

Control of pest by the routine use of chemical insecticides are practiced in many countries in dry fish but it creates several problems in agro-ecosystem such as direct toxicity to beneficial insects, fishes and man, pesticides resistance, health hazard and increased environmental and social costs (Pimental *et al.*, 1980). Sometimes persistent pesticides

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accumulate in the higher food chain of both wild life and human and become concentrated by bio-magnification (Metcalf and Luckmann, 1975).

Botanical insecticides are considered to be the alternative to synthetic chemical pesticides since these compounds are biodegradable and less persistent in the environment (Golob and Webley, 1980; Talukder and Howse, 1993, 1995). Plants are the rich source of insecticidal compounds and the effectiveness of these compounds has been demonstrated against many stored product insects (Xie *et al.*, 1995). Extracts of the neem (*Azadirachta indica*), red chili (*Capsicum frutescens*) and garlic (*Allium sativum*) have yielded botanical insecticides which have been tested extensively in agricultural situations (Sighamony, 1986). Since these products have been shown to be effective against many pests, including beetle and almost exclusively phytophagous, they might also be a safe alternative to synthetic chemicals for the control of storage beetle and mites of dry fish, such as *Dermestes sp.* and *Necrobia sp.* Considering this, the suitability of neem, garlic and red chili in terms of insecticidal and repellent effects against common dry fish beetle and mite was tested.

MATERIALS AND METHODS

Collection and rearing of insect

The test insects, *Necrobia sp.* adult and *Dermestes sp.* larvae were collected from the dry fish markets of Mymensingh town and were maintained in the Postgraduate Laboratory of the Department of Fisheries Technology, BAU. The insects were reared on crushed dry fish in plastic jars.

Collection and processing of plant materials

Neem leaves were collected from the surroundings of BAU campus, Mymensingh. After bringing them to the laboratory, they were washed in running water, kept in the shade for air drying and then dried in the oven at 60°C to gain constant weight. Garlic and red chili were purchased from the local market. Both garlic and red chili were dried in oven at 50°C for 2-3 hours to remove moisture.

Preparation of plant dust and extracts

Plant dusts were prepared by pulverizing the dried plant materials with the help of a grinder. The dusts were passed through a 25-mesh sieve to obtain a fine and uniform dust. The dusts were preserved in airtight Zip-lock® polythene bags till their use in extract preparation.

Ten gram of each of the dusts were taken in a 500 ml beaker and separately mixed with 100 ml acetone. Then the mixture was stirred for 30 minutes by a magnetic stirrer (at 3000 rpm) and left for 24 hours. The mixture was filtered through a fine cloth and again through the filter paper (Whatman No. 1). The filtered materials were taken into a round bottom flask and condensed by evaporation of the solvent in a water bath at 45°C.

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Evaporation was done to make the volume to 10 ml. Condensed extracts were preserved in tightly corked labeled bottles and stored in a refrigerator until their use for insect bioassays.

Preparation of stock solution

Stock solutions of plant extracts were prepared separately by diluting the condensed acetone extracts. Different concentrations of each category of plant extract viz., 10%, 15%, 20% for *Necrobia sp.* adult and 60%, 80%, 100% for *Dermestes sp.* larvae were prepared by dissolving the stock solutions in acetone prior to insect bioassay.

De-infestations of dry fish

Sun dried fish were kept in an oven at 45°C for 2-3 hours, packed in polythene bag and sealed to avoid future infestation.

Insect bioassays

Insect bioassay was conducted in the Microbiology Laboratory of the Department of Fisheries Technology, BAU at 27-30°C to determine direct toxicity against *Necrobia sp.* adult and *Dermestes sp.* larvae.

Direct toxicity test

Direct toxicity was determined according to the method of Talukder and Howse (1993). Three different concentrations of each plant extracts (for *Necrobia sp.* adult 10%, 15% and 20% and for *Dermestes sp.* larvae 60%, 80% and 100%) were prepared with respective solvents. Then 5 μ l of prepared solution was applied to the dorsal surface on the thorax of each insect for *Necrobia sp.* adult and 30 μ l for *Dermestes sp.* larvae by using a micropipette. Ten insects (five males and five females) per replication were treated and each treatment was replicated 3 times. In addition, the same numbers of insects were treated with only basal solvent as control. After treatment, the insects were transferred into 9 cm diameter petridishes (10 insects/petridish) containing dried fish. Insect mortalities were recorded at 24, 48 and 72 hours after treatment (HAT). Original data were corrected by Abbott's (1987) formula.

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

Where,

P = Percentage of corrected mortality

P = Observed mortality (%)

C = Control mortality (%)

Statistical analysis

The experimental data were statistically analysed by completely randomized design (factorial CRD) using MSTAT statistical software in a microcomputer. The mean values

were compared by Duncan's Multiple Range Test (Duncan, 1951). The lethal concentration 50 (LC_{50}) values were calculated by using probit analysis.

RESULTS AND DISCUSSION

Direct toxic effect

Application of red chili to *Necrobia sp.* adult and *Dermestes sp.* larvae obtained the highest initial mortality due to the presence of piperine (Scngypt and Ray, 1987). Neem also showed satisfactory rapid mortality and proved to be one of the most effective bio-insecticide. Within two days of the application to the insect, red chili produced the highest mortality. Garlic extract did not act as rapidly as red chili and neem, but produced some mortality after two days.

Toxicity on Necrobia sp. adult

The results of direct toxic effects of different plant extracts against *Necrobia sp.* adult have been presented in Tables 1-3. The mortality percentage of this insect at 24, 48 and 72 hours after treatment (HAT) indicated that red chili extracts possessed the highest mortality. The order of toxicity of the three plant extracts on *Necrobia sp.* adult were, red chili > neem > garlic. It was found that the mortality was directly proportional to the concentration of plant extract. The interaction effects of dose and time had significant effect on the mortality of *Necrobia sp.* adult. Their average values were also significant.

Name of the		Mortality percentage			
plants	24 HAT	48 HAT	72 HAT		
Neem	29.167	39.167	44.167	37.500ь	
Red chili	35.833	45.000	53.333	44.722 ^a	
Garlic	19.167	25.833	38.333	27.778°	

 Table 1. Direct toxic effect of different plant extracts on Necrobia sp. adult in treated dried fish at different HAT (interaction of plant and time)

HAT = Hours after treatment

Within column values followed by different superscripts are significantly different (P<0.01).

Toxicity on Dermestes sp. larvae

The efficacy of plant extracts was also similar in *Dermestes sp.* larvae as in case of *Necrobia sp.* adult (Table 4-6). The order of toxicity of the three plant extracts on *Dermestes sp.* larvae were, red chili > neem > garlic. It was also found that the mortality was directly proportional to the concentration of plant extract.

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Table 2. Direct toxic effect of doses of different plant extracts	s on Necrobia sp. adult in
treated dried fish at different HAT (interaction of dose a	nd time)

Doses (%)		Average		
	24 HAT	48 HAT	72 HAT	
Control	16.67 g	16.67g	20.00fg	17.78 ^d
10	26.67ef	36.67 ^d	45.56 ^c	36.29°
15	33.33 ^{de}	44.44 ^c	53.33 ^b	43.70 ^b
20	35.56 ^d	48.89bc	62.62 ^a	48.89ª

HAT = Hours after treatment

Within column values followed by different superscripts are significantly different (P<0.01)

 Table 3. Direct toxic effect of different plant extracts at different dose level on *Necrobia sp.*

 adult in treated dried fish at different HAT (interaction of plant, dose and time)

Name of the plants	Doses (%)	Mortality percentage			Average
		24 HAT	48 HAT	72 HAT	
Control	-	16.67	16.67	20.00	17.78 ^e
Neem	10	26.67	36.67	43.33	35.56 ^d
	15	36.67	46.67	53.33	45.56 ^c
	20	36.67	56.67	60.33	51.11 ^{bc}
Red chili	10	36.67	43.33	53.33	44.44 ^c
	15	43.33	56.67	63.33	54.44 ^b
	20	46.67	63.33	76.67	62.22 ^a
Garlic	10	16.67	30.00	40.00	28.89 ^d
	15	20.00	30.00	43.33	31.11 ^d
	20	23.33	26.00	50.00	33.33 ^d

HAT = Hours after treatment

Within column values followed by different superscripts are significantly different (P<0.01)

Table 4. Direct toxic effect of different plant extracts on *Dermestes sp.* larvae in treated dried fish at different HAT (interaction of plant and time)

Name of the plants	Ν	Average		
	24 HAT			
Neem	31.67	35.83	40.00	35.83 ^b
Red chili	45.00	47.50	50.83	47.78 ^a
Garlic	27.50	35.83	38.33	33.89 ^b

HAT = Hours after treatment

Within column values followed by different superscripts are significantly different (P<0.01)

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Probit analysis of direct toxic effect

The results of the probit analysis for the estimation of LC_{50} values and their 95% fiducial limits and the slope of regression lines at 24, 48 and 72 HAT for the mortality of both *Necrobia sp.* adult are presented in Tables 7-8.

 Table 5. Direct toxic effect of doses of different plant extracts on *Dermestes sp.* larvae in treated dried fish at different HAT (interaction of dose and time)

Doses (%)	1	Average		
	24 HAT	48 HAT	72 HAT	
Control	10.00	10.00	10.00	10.00 ^c
60	37.78	45.56	48.89	44.07 ^b
80	40.00	47.78	51.11	46.29 ^b
100	51.11	55.56	62.22	56.29 ^a

HAT = Hours after treatment

Within column values followed by different superscripts are significantly different (P<0.01)

Table 6. Direct toxic effect of different plant extracts at different dose level on *Dermestes sp.* larvae in treated dried fish at different HAT (interaction of plant, dose and time)

Name of the plants	Doses (%)	Mortality percentage			Average
		24 HAT	48 HAT	72 HAT	
Control	-	10.00	10.00	10.00	10.00 ^d
Neem	60	33.33	36.67	40.00	36.67c
	80	33.33	40.00	46.67	40.00 ^c
	100	50.00	56.67	63.33	56.67ab
Red chili	60	50.00	56.7	60.00	55.56 ^{ab}
	80	56.67	60.00	60.00	58.89a
	100	63.33	63.33	73.33	66.67 ^a
Garlic	60	30.00	43.33	46.67	40.00 ^c
	80	30.00	43.33	46.67	40.00c
	100	40.00	46.67	50.00	45.56 ^{bc}

HAT = Hours after treatment

Within column values followed by different superscripts are significantly different (P<0.05)

Direct toxicity on Necrobia sp. adult

The LC_{50} values of neem, red chili and garlic at 24 HAT (Table 7) indicated that red chili was the most toxic and garlic was the least toxic plant. Neem showed 0.85%, red chili 0.78% and garlic 1.68% toxicity at 24 HAT. They also maintained their toxicity

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accordingly when the LC_{50} values were compared at 48 HAT (neem 0.73%, red chili 0.29% and garlic 1.11%) and 72 HAT (neem 0.56%, red chili 0.03% and garlic 0.90%).

Direct toxicity on Dermestes sp. larvae

The LC₅₀ values of three plant extracts were - 0.69%, 0.60%, 1.15% toxicity at 24 HAT; 0.52%, 0.48%, 0.57% at 48 HAT and at 72 HAT were 0.44%, 0.36%, 0.46% for neem, red chili and garlic respectively which indicated that red chili was the most toxic and garlic was the least toxic among the three plants (Table 8).

 Table 7. Relative toxicity (by probit analysis) of different plant extracts treated against

 Necrobia sp. adult at 24, 48 and 72 HAT

Name of the extracts	No. of the insect used	LC_{50} values	95% fiducial limit	χ ² values	Slope ± DE
24 HAT					
Neem	10	0.85	-5.07-4.93	0.003	2.28 ± 0.81
Red chili	10	0.78	-5.12-4.90	0.111	2.60 ± 0.81
Garlic	10	1.682	-4.92-5.37	0.060	1.30 ± 0.83
48 HAT					
Neem	10	0.73	-5.11-4.84	0.0008	1.90 ± 0.80
Red chili	10	0.29	-5.58-4.51	0.0007	0.79 ± 0.80
Garlic	10	1.11	-5.02-5.11	0.235	1.88 ± 0.82
72 HAT					
Neem	10	0.56	-5.19-4.69	0.005	0.38 ± 0.79
Red chili	10	0.03	-6.67-3.78	0.011	0.41 ± 0.84
Garlic	10	0.90	-5.06-4.97	0.164	2.22 ± 0.81

HAT= Hours after treatment

Values were based on one solvent, three concentrations and three replications of 10 insects each χ^2 = Goodness of fit

Probit regression lines

The probit regression lines for the effect of three different plant extracts are shown in Figs. 1-3. The insect mortality rate showed positive correlation with the doses in all cases. The probit regression lines for the effects of three different plant extracts on *Necrobia sp.* adult and *Dermestes sp. larvae* showed a clear linear relationship between probit-mortality and the doses used for them.

The calculated probit regression equation of neem extracts for *Necrobia sp.* adult (Fig. 1) at 24 HAT was, Y = 2.0111x + 1.2546, at 48 HAT was Y = 2.3314x + 2.0562, and for 72 HAT was Y = 2.8836x + 2.1309. It was indicated from above equations that mortality rate increased with the increase of concentration and time.

The calculated probit regression equation of red chili extracts for *Necrobia sp.* adult (Fig. 2) at 24 HAT was, Y = 2.4726x + 1.6355, at 48 HAT was Y = 2.9682x + 2.2452, and for 72 HAT

was Y = 3.3168x + 2.7793. The equations indicate that mortality rate increases with increase of concentration and time.

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Name of the	No. of the	LC ₅₀ values	95% fiducial	χ^2	Slope ± DE
extracts	insect used		limit	values	
24 HAT					·
Neem	10	0.69	-5.16-4.85	0.010	2.31 ± 0.81
Red chili	10	0.60	-5.20-4.77	0.002	1.52 ± 0.80
Garlic	10	1.15	-4.93-5.06	0.002	1.16 ± 0.81
48 HAT					
Neem	10	0.52	-6.27-5.71	0.208	4.98 ± 0.96
Red chili	10	0.48	-7.02-6.39	0.419	5.48 ± 1.08
Garlic	10	0.57	-5.38-4.90	0.045	2.84 ± 0.38
72 HAT					
Neem	10	0.44	-5.43-4.73	0.174	1.54 ± 0.82
Red chili	10	0.36	-5.44-4.56	0.0005	0.76 ± 0.81
Garlic	10	0.46	-7.81-7.15	0.062	5.88 ± 1.21

Table 8. Relative toxicity (by probit analysis) of different plant extracts treated againstDermestes sp. larvae at 24, 48 and 72 HAT

HAT = Hours after treatment

Values were based on one solvent, three concentrations and three replications of 10 insects each $X^2 =$ Goodness of fit

The calculated probit regression equation of garlic extracts for *Necrobia sp.* adult (Fig. 3) at 24 HAT was, Y = 2.1379x - 0.0027, at 48 HAT was Y = 2.8152x + 0.1879, and for 72 HAT was Y = 4.644x + 0.691. It was again indicated from the above equations that mortality rate increased with increase of concentration and time. All three cases the highest mortality was at 72 HAT.

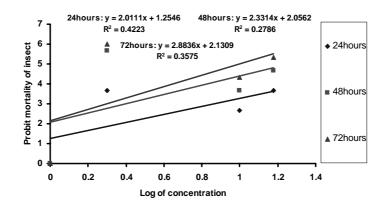


Fig. 1. Relationship between probit mortality and log doses of neem extracts on Necrobia *sp.* adult at 24, 48 and 72 hours after treatment

The probit regression lines for the effects of three plant extracts on *Dermestes sp.* larvae also showed similar distinct linear relationship between probit mortality and the doses used (Figs. 4, 5 and 6). Highest mortality was also found at 72 HAT in all three extracts.

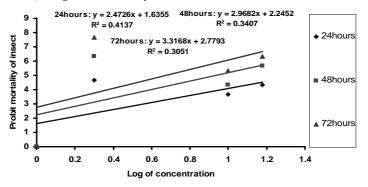


Fig. 2. Relationship between probit mortality and log doses of red chili extracts on Necrobia *sp.* adult at 24, 48 and 72 hours after treatment

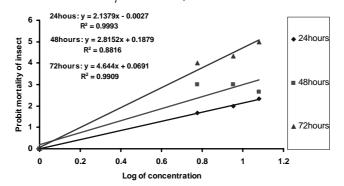


Fig. 3. Relationship between probit mortality and log doses of garlic extracts on Necrobia *sp.* adult at 24, 48 and 72 hours after treatment

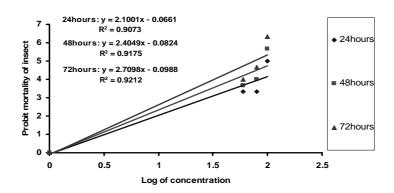


Fig. 4. Relationship between probit mortality and log doses of neem extracts on *Dermestes sp.* larvae at 24, 48 and 72 hours after treatment

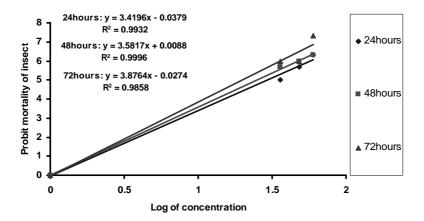


Fig. 5. Relationship between probit mortality and log doses of red chili extracts on *Dermestes sp.* larvae at 24, 48 and 72 hours after treatment

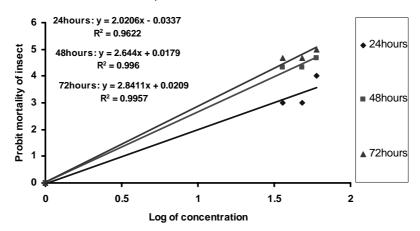


Fig. 6. Relationship between probit mortality and log doses of garlic extracts on *Dermestes sp.* larvae at 24, 48 and 72 hours after treatment

CONCLUSION

For *Necrobia sp.* adult LC₅₀ values of red chili (0.78%), neem (0.85%) and garlic (1.68%) at 24 HAT indicated that red chili had the highest toxic effect among these three herbs. Red chili possessed the highest toxic effect at 48 HAT (0.29%) and 72 HAT (0.03%). For larvae of *Dermestes sp.* the LC₅₀ values of red chili was 0.60%, of neem was 0.69% and of garlic was 1.15% at 24 HAT, again indicating the red chili as the most toxic plant. All the three plant extracts had repellent and toxic effects on dried fish beetle and mite. Red chili extract was found to be the most toxic, while neem to be the most repellent.

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