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Efficacy of some botanical pesticides (neem, garlic and red chili) against dried 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 toxic effects against a 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 percentage of *Necrobia sp*. (adult) at 24, 48 and 72 hours after treatment (HAT) indicated that red chili extract possessed the highest toxic effect followed by neem and garlic. Mortality percentage was found to be directly proportional to the level of concentration of plant extracts. For *Necrobia sp*. (adult) LC_{50} values of red chili was 0.78%, neem 0.85% and of garlic was 1.68% at 24 HAT. Red chili was found to be the most toxic herb among these three. Red chili also possessed the highest toxic effect at 48 HAT (0.29%) and 72 HAT (0.03%). For the larvae of *Dermestes sp.*, the LC_{50} values of red chili was 0.60%, neem 0.69% and garlic 1.15% at 24 HAT, again indicating that red chili was the most toxic plant.
Key Words: Neem, garlic, red chili, toxic effects, dried fish, probit analysis

Introduction

In Bangladesh dried fishes are attacked by several species of dermestid beetle and mites' viz., *Dermestes maculatus*, *D. frischii*, *D. ater* and mite *Necrobia rufipes* during storage. 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 dried 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 (Meynell, 1978). Control of pest by the routine use of chemical insecticides are practiced in dried 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 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 the alternative to synthetic chemical pesticides since these compounds are biodegradable and less persistent in the environment. 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. Since these products have been shown to be effective against many pests, including many beetle pests, mainly agricultural and almost exclusively phytophagous, they might also be a safe alternative to synthetic chemicals for the control of storage beetle and mites of dried fish such as *Dermestes sp.* and *Necrobia sp*. Considering this, the efficacy of neem, garlic and red chili were evaluated against common dried fish beetle and mite were tested.

Materials and Methods

Collection and rearing of insect: The test insect *Necrobia sp.* adult and *Dermestes sp.* larvae were collected from dried fish markets of Mymensingh town and were maintained in the Postgraduate Laboratory of the Department of Fisheries Technology, BAU. The insects were reared on dried 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 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 60°C to remove moisture.

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

Preparation of plant extracts: Ten gram of each of the dusts were taken in a 500 ml beaker separately and 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 temperature. Evaporation was done to make the volume of 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 extracts acetone. 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.

Disinfestations of dried 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 to 30°C temperature 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 5µl of prepared solution was applied to the dorsal surface of the thorax of each insect for *Necrobia sp.* adult and 30µl for *Dermestes sp.* larvae by using a micropipette. Ten insects (five males & five females) 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 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 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 mortalities 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 the Tables 1-3. The order of toxicity of the three plant extracts on *Necrobia sp.* were, red chili > neem > garlic. It was found that the mortality percentage was directly proportional to the level of concentration of plant extract.

In Table 1 (interaction of plant and time) average mortality percentage of insect was 44.72% for red chili extract, 37.50% for neem extract and 27.78% for garlic extract. So, red chili was found to be more toxic. The interaction effects of plant and time has no significant effect on the mortality of *Necrobia sp*., except their average values.

Table 2 (interaction of dose and time) shows that average mortality percentage of insect was 36.29% for 10% extract, 43.70 for 15% and 48.89 for 20% extract. So, 20% dose was more toxic here. The interaction effects of dose and time had significant effect on the mortality of *Necrobia sp*. Their average values were also significant.

Table 3 (interaction of plant, dose and time) shows that the highest average mortality percentage of insect was 62.22% for 20% red chili extract. The lowest mortality percentage of insect was 28.89% for 10% garlic extract. The interaction effects of plant, dose and time has no significant effect on the mortality of *Necrobia sp.* except their average values.

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)

Name of the plants	Mortality Percentage			Average
	24 HAT	48 HAT	72 HAT	
Neem	29.167	39.167	44.167	37.500^{b}
Red chili	35.833	45.000	53.333	44.722^{a}
Garlic	19.167	25.833	38.333	27.778^{c}

HAT = Hours after treatment, Within column values followed by different superscripts are significantly different ($p<0.01$)

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

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

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	Mortality Percentage			Average
	24 HAT	48 HAT	72 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).

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 (%)	Mortality Percentage			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).

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. From Table 4 (interaction of plant and time) average mortality percentage of insect was found 47.53% for red chili extract, 35.83% for neem extract and 33.89% for garlic extract. Red chili extract was found to be more toxic again. The interaction effects of plant and time had no significant effect on the mortality of *Dermestes sp.* (larvae) except their average values.

In Table 5 (interaction of dose and time) average mortality percentage of insect was 44.07% for 60% extract, 46.29% for 80% and 56.29% for 100% extract. Here as a usual case 100% dose was more toxic. The interaction effects of dose and time had no significant effect on the mortality of *Dermestes sp.* (larvae) except their average values.

In Table 6 (interaction of plant, dose and time) highest average mortality percentage of insect was 66.67% for 100% red chili extract. The lowest mortality percentage of insect was 40% for 60% garlic extract. The interaction effects of plant, dose and time had no significant effect on the mortality of *Dermestes sp.* (larvae) except their average values.

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 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]
	60	33.33	36.67	40.00	36.67[c]
Neem	80	33.33	40.00	46.67	40.00[c]
	100	50.00	56.67	63.33	56.67[ab]
	60	50.00	56.7	60.00	55.56[ab]
Red chili	80	56.67	60.00	60.00	58.89[a]
	100	63.33	63.33	73.33	66.67[a]
	60	30.00	43.33	46.67	40.00[c]
Garlic	80	30.00	43.33	46.67	40.00[c]
	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).

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
		72HAT			
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±.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.

Table 8. Relative toxicity (by probit analysis) of different plant extracts treated against *Dermestes sp*. larvae 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	0.69	-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
		72HAT			
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

[HAT= Hours after treatment, Values were based on one solvent, three concentrations and three replications of 10 insects each, χ^2= Goodness of fit.

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 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%).

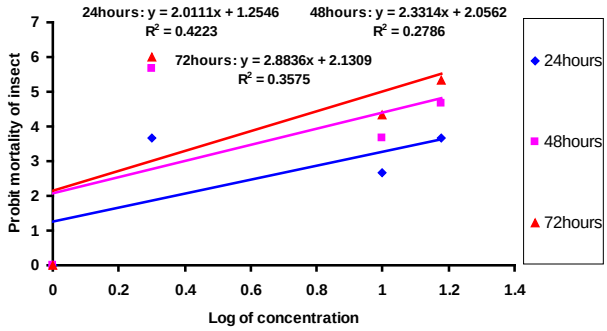

Fig. 1. Relationship between probit mortality and log doses of neem extracts on Necrobia *sp*. adult at 24, 48 and 72 hours after treatment

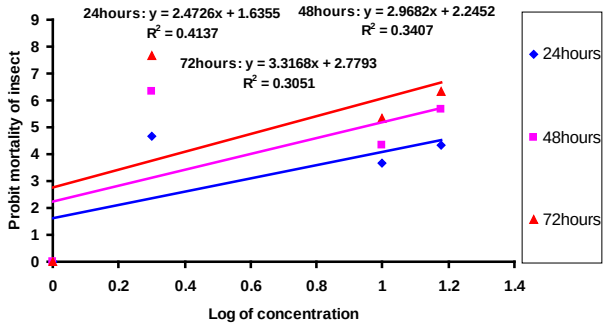

Fig. 2. Relationship between probit mortality and log doses of red chili extracts on Necrobia *sp*. adult at 24, 48 and 72 hours after treatment

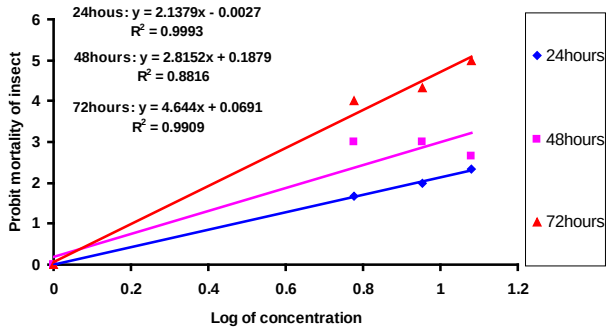

Fig. 3. Relationship between probit mortality and log doses of garlic extracts on Necrobia *sp*. adult at 24, 48 and 72 hours after treatment

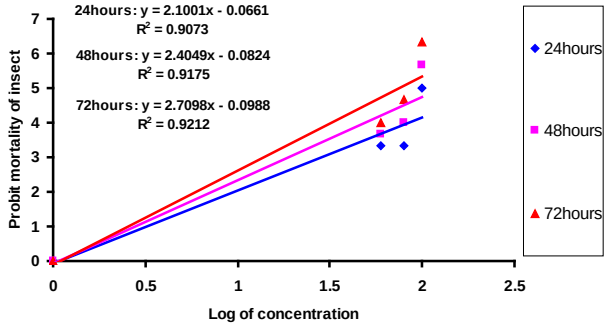

Fig. 4 Relationship between probit mortality and log doses of neem extracts on *Dermestes sp*. larvae at 24, 48 and 72 hours after treatment

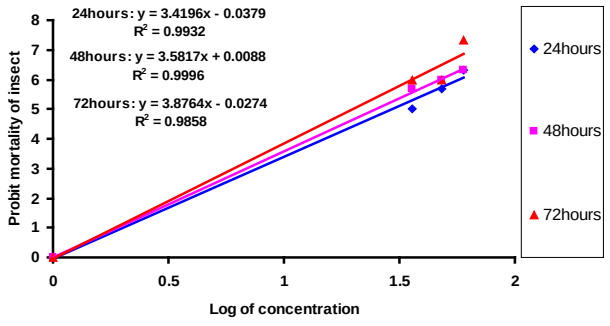

Fig. 5. Relationship between probit mortality and log doses of red chili extracts on *Dermestes sp.* larvae at 24, 48 and 72 hours after treatment

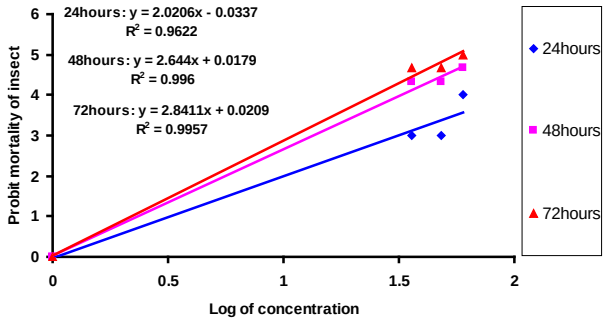

Fig. 6. Relationship between probit mortality and log doses of garlic extracts on *Dermestes sp.* larvae at 24, 48 and 72 hours after treatment

Direct toxicity on *Dermestes sp.* larvae: The LC_{50} 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).

Probit regression lines: The probit regression lines for the effect of three different plant extracts are shown in Fig. 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. 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.

Regression lines on *Dermestes sp.* (larvae): 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 (Fig 4, 5 and 6). The calculated probit regression equations of neem extracts for *Dermestes sp.* (larvae) at 24 HAT were, Y = 2.1001x – 0.0661, at 48 HAT were Y = 2.4049x – 0.0824, and for 72 HAT were Y = 2.7098x – 0.0988, which indicates that mortality rate increases with increase of concentration and time. Highest mortality was at 72 HAT (Fig. 4).

The calculated probit regression equations of red chili extracts for *Dermestes sp.* (larvae) at 24 HAT were, Y = 3.4196x – 0.0379, at 48 HAT were Y = 3.5817x + 0.0088, and for 72 HAT were Y = 3.8764x – 0.0274. Equations indicate that mortality rate increases with increase of concentration and time. Highest mortality was at 72 HAT (Fig. 5).

The calculated probit regression equations of garlic extracts for *Dermestes sp.* (larvae) at 24 HAT (Fig. 6) were, Y = 2.0206x – 0.0337, at 48 HAT were Y = 2.644x + 0.0179, and for 72 HAT were Y = 2.8411x + 0.0209, which also indicate the same results.

For *Necrobia sp.* adult LC_{50} values of red chili (0.78%), neem (0.85%) and garlic (1.68%) at 24 HAT indicated that red chili was the most toxic 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.* larvae, the LC_{50} 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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