Progress. Agric. 20(1 & 2) : 133 – 142, 2009 ISSN 1017-8139

EFFECTIVENESS OF NEEM, GARLIC AND RED CHILI AGAINST ADULT DERMESTID BEETLE IN SUN DRIED FISH

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ABSTRACT

The suitability of neem (*Azadirachta indica*), garlic (*Allium sativum*) and red chili (*Capsicum frutescens)* in repelling insects in dried fish during processing and storage was determined. Their acetone extracts were used to evaluate toxic effects against adult dermestid beetle, *Dermestes* sp. The concentration of plant extracts were 60%, 80% and 100% for adult *Dermestes* sp. The LC_{50} values of neem, garlic and red chili were 1.81%, 4.85% and 1.11%, respectively at 24 hours after treatment (HAT), indicating that red chili was the most toxic plant. Red chili possessed the highest toxic effect at 48 HAT (0.87%) and 72 HAT (0.03%). It was observed that all of the three plant extracts had repellent action on adult *Dermestes* sp. The highest mean repellency was observed in neem extract (62.67%) followed by red chili (38.44%) and garlic (31.11%). Repellent effects increased with the concentration of plant extracts.

Key words : Botanical pesticides, Dermestid beetle, Toxic effects, Dried fish, Repellent effects

INTRODUCTION

About 20% of the total fish caught annually in Bangladesh are sun dried and mostly consumed by the domestic market. One of the major problems associated with the sun-drying of fish is the infestation of the products by the blow fly and beetle larvae (Nowsad, 2005). In tropical climates under highly humid conditions, heavy infestation of unsalted dry fish by beetles may cause up to 30 % loss of the products (Bala and Mondol, 2001). The other problems markedly evident with dried fish are the contamination during different stages of handling and the indiscriminate use of various pesticides (Nowsad, 2005). Dried fish contaminated by both insects and insecticides comprises about 60% of the total dried products that is considered not fit for human consumption (Neuschler, 1998).

Dried fish is readily attacked by several species of dermestid beetle, including *Dermestes maculatus*, *D. frischii* and *D. ater*. These insects are generally associated with dried fish especially during storage, transportation and marketing (Don-Pedro, 1989). Use of unsafe insecticides and their excessive dosages in dried fish create serious health problems to the consumers (Khan *et al.*, 2002). DDT has been found to be in excessive quantity in different dried fish products in Bangladesh, the level of which are much higher than the FAO

allowable limit of 0.5 mg/kg (Khan *et al.*, 2002) or FDA approved tolerance, action and guidance level of 5 ppm (FDA, 1996).

Botanical pesticides are generally considered to be safe when compared to synthetic chemical pesticides; they are considered to be non-toxic to mammals, birds and fish (Ascher, 1993) and cause less disturbance to ecosystems than chemical insecticides (Sundaram, 1996). There has been no evidence of the development of resistance of crude neem products to date, although there has been some evidence of the development of resistance of purified azadirachtin (Jilani *et al.,* 1990; Ascher, 1993). Garlic and chili are also considered to be safe for human. Nonetheless all the three botanical pesticides have extra health benefit to the man (Ascher, 1993). Considering such advantages, the suitability of their use in dried fish was tested through investigating the toxic and repellent effects of neem, red chili and garlic against a common dried fish beetle *Dermestes* sp.

MATERIALS AND METHODS

The present study was conducted in the Microbiology Laboratory of the Department of Fisheries Technology, Bangladesh Agricultural University (BAU). Selected plants were tested to determine their toxic and repellent effects on the beetles and mites of various dried fish purchased from the fish markets.

Collection and rearing of insect

The test insect species, *Dermestes* sp. (adult) was collected from dried fish markets of Mymensingh, Bangladesh. The insects were maintained in the postgraduate laboratory of Faculty of Fisheries, BAU at 27-30°C. The insects were reared with dried fish in plastic jars.

Collection of plant materials

Neem leaves were collected from the BAU, Mymensingh. Red pepper and garlic were purchased from K. R. Market, BAU, Mymensingh. After bringing them to the laboratory, they were washed in running water. The plant materials were first kept in shade for air drying and then they were dried in a hot air electric oven at 60°C to gain constant weight.

Preparation of plant dust

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

Preparation of plant extracts

Ten gram of each category of the dusts were taken in a 500ml 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 to stand for 24 hours. The mixture was then filtered through a fine cloth and again through a filter paper (Whatman No. 1). The filtered materials were taken into a round bottom flask and condensed by evaporation of solvent in a water bath at 45°C. 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 with acetone. Different concentrations like 60%, 80% and 100% of each category of plant extract 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 Faculty of Fisheries at 27 to 30°C to determine direct toxicity and repellency against adult *Dermestes* sp.

Direct toxicity test

Direct toxicity was determined according to the method of Talukder and Howse (1993). Three different concentrations of each plant extracts (60, 80 and 100%) were prepared with respective solvents. Then 30 µl of prepared solution was applied to the dorsal surface of the thorax of each insect by touching with 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 HAT. Original data were corrected by Abbott's (1987) formula as follows :

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

Where,

P = Percentage of corrected mortality
P′ = Observed mortality (%)
C = Control mortality (%)

Repellency test

Repellency test was conducted according to the method of Talukder and Howse (1994). Petridishes with semi powdered dried fish were divided into two parts, treated and untreated with a sharp dividing line at the centre. With the help of a pipette, 1 ml

solution of each plant extract was applied to one half of the dried fishes. The treated half was then air-dried. Ten insects (five males and five females) were released at the centre dividing line of each petridish and a cover was placed on the petridish. For each plant extract and each dose, three replications were used. Then the insects present on each portion were counted at hourly intervals up to fifth hour.

The data were expressed as percentage repulsion (PR%) by the following formula :

PR (%) = (NC-50) x 2

Where,

NC = The percentage of insects present in the control half.
Positive (+) values expressed repellency and negative (-) values attractency.

Data (PR%) were analyzed using analysis of variance (ANOVA). The average values were then categorized according to the following class according to Mc Donald *et al.*, (1970).

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

Statistical analysis

The experimental data were analysed by a completely randomized design (factorial CRD) using MSTAT statistical software. The mean values were compared by Duncan's Multiple Range Test (DMRT) (Duncan, 1951). The LC_{50} values were calculated by using probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Direct toxic effect

Direct toxic effects of different plant extracts on adult *Dermestes* sp. in dried fish at different (HAT have been shown in Table 1 and Table 2. Application of red chili to *Dermestes* sp. produced the highest initial mortality. Scngypt and Ray (1987) found similar results with red chili that might be due to the presence of piperine. The order of toxicity of the three plant extracts on *Dermestes* sp. were, red chili > neem > garlic. It was found that the mortality percentage was also directly proportional to the level of concentration of plant extract. In Table 1 average mortality percentage was 65.56% for red

chili extract, 49.44% for neem extract and 28.33% for garlic extract. In Table 2 average mortality percentage was 50.741% for 60% dose, 63.70% for 80% dose and 65.56% for 100% dose of extract. So naturally, 100% dose was more toxic. In Table 3 highest mortality percentage was found at 93.33% for 100% red chili extract. Lowest average mortality percentage was found at 25.56% for 60% garlic extract. The interaction effects of plant, dose and time had no significant effect on the mortality of adult *Dermestes* sp. except their average values.

Table 1. Direct toxic effect of different plant extracts on adult *Dermestes* sp. 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	41.67	50.00	56.67	49.44^{b}
Red chili	61.67	65.83	69.17	65.56^{a}
Garlic	19.17	28.33	37.50	28.33^{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 adult *Dermestes* sp. 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	13.33	11.11^{c}
60	41.11	52.22	58.89	50.74^{b}
80	55.56	64.44	71.11	63.70^{a}
100	56.67	65.56	74.44	65.56^{a}

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

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 *Dermestes sp.* are presented in Table 4. The LC_{50} values of neem, red chili and garlic showed 1.81, 1.11, 4.85% toxicity at 24 HAT; 1.77, 0.87, 2.30% at 48 HAT and 1.05, 0.03 and 1.87% at 72 HAT respectively. From the above probit results, it was clear that all tested plants were more or less effective for controlling adult *Dermestes* sp. but red chili was the most effective plant.

Table 3. Direct toxic effect of different plant extracts at different dose level on adult *Dermestes* sp. 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	13.33	11.11[d]
Neem	60	50.00	60.00	70.00	60.00[b]
	80	53.33	63.33	70.00	62.22[b]
	100	53.33	66.667	73.33	64.44[b]
Red chili	60	60.00	66.67	73.33	66.67[b]
	80	86.7	93.33	93.33	91.11[a]
	100	90.00	93.33	96.67	93.33[a]
Garlic	60	13.33	30.00	33.33	25.56[c]
	80	26.67	36.67	50.00	37.78[c]
	100	26.67	36.67	53.33	38.89[c]

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

Table 4. Relative toxicity of different plant extracts treated against adult *Dermestes* sp. 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	1.81	-4.88-5.40	0.089	1.18±0.83
Red chili	10	1.11	-4.89-4.99	0.009	0.35±0.79
Garlic	10	4.85	-4.99-6.37	0.0002	1.06±0.92
		48 HAT			
Neem	10	1.77	-5.40-5.90	0.162	2.19±0.91
Red chili	10	0.87	-4.69-5.08	0.084	2.36±0.81
Garlic	10	2.30	-4.74-3.58	0.026	0.85±0.82
		72 HAT			
Neem	10	1.05	-4.96-4.99	0.014	1.12±0.80
Red chili	10	0.03	-6.67-3.38	0.011	-0.41±0.84
Garlic	10	1.87	-4.69-5.23	0.009	0.36±0.80

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 insect mortality rate showed positive correlation with the doses in all cases. The probit regression lines for the effects of three different plant extracts showed a clear linear relationship between probit-mortality and the doses used for them. The calculated probit regression equation of neem extracts at 24 HAT was Y = 2.7467x + 0.0155, at 48 HAT was Y = 3.3411x + 0.0046 and for 72 HAT was Y = 3.7339x + 0.0301 (Fig. 1). For red chili extracts equations were at 24 HAT: Y = 4.8255x – 0.1342, at 48 HAT, Y = 5.1372x – 0.1084 and at 72 HAT Y = 5.3146x – 0.0808 (Fig. 2). For garlic extracts equations were: at 24 HAT, Y = 1.3863x – 0.0717, at 48 HAT Y = 2.0725x – 0.0154 and for 72 HAT Y = 2.805x – 0.095 (Fig. 3). All of these equations indicated similar results that mortality rate increased with the increased of concentration and time. Highest mortality was at 72 HAT.

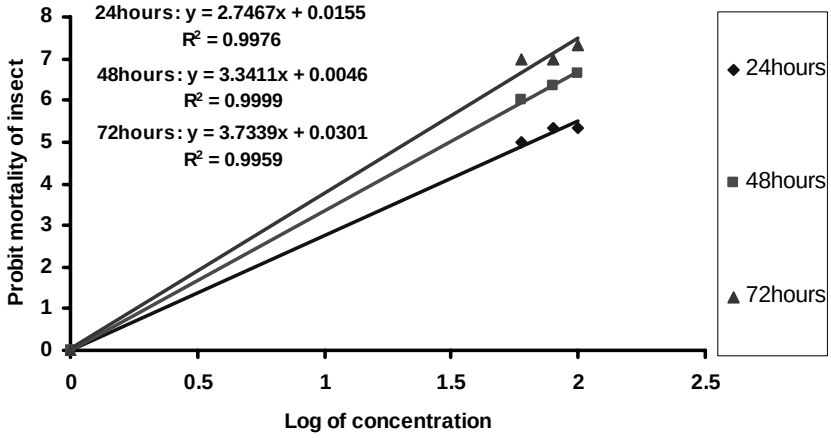

Fig. 1. Relationship between probit mortality and log doses of neem extracts on adult *Dermestes* sp. at 24, 48 and 72 hours after treatment

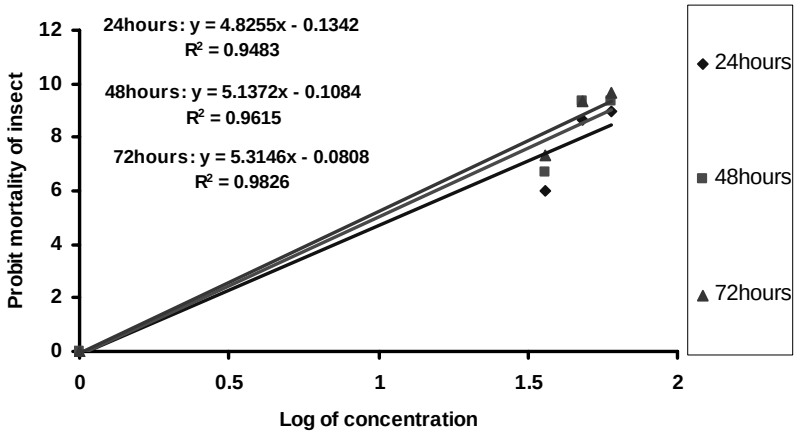

Fig. 2. Relationship between probit mortality and log doses of red chili extracts on adult *Dermestes* sp. at 24, 48 and 72 hours after treatment

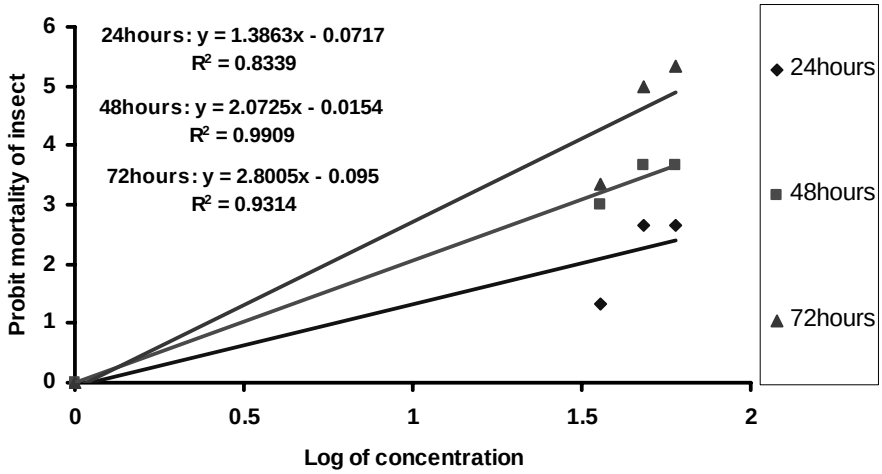

Fig. 3. Relationship between probit mortality and log doses of garlic extracts on adult *Dermestes* sp. at 24, 48 and 72 hours after treatment

Repellent effect

Table 5 shows the repellent effects of different plant extracts. Among the three plant extracts tested, neem showed the highest (62.67%) mean repellency effect followed by red chili (38.44%) and garlic (31.11%). On the basis of mean repellency rate the repellency class for red chili was II, for neem was IV and for garlic was II (Table 5). The repellency effects of different plant extracts at different dose level on *Dermestes* sp. are presented on Table 6. The highest (56.66%) mean repellency was found at 100% concentration level and the lowest (31.56%) was found at 60% concentration level. Table 7 showed that the highest (82.67%) mean repellency was found with 100% neem extract and the lowest (20%) mean repellency was found with 60% garlic extract. From the above results, it was found that neem extracts showed more repellent effect than other plant extracts.

Table 5. Repellent effect of different plant extracts on *Dermestes* sp. in treated dried fish at different HAT (interaction of plant and time)

Name of the plants	Repellency rate					Mean repellency	Repellency class
	1 HAT	2 HAT	3 HAT	4 HAT	5 HAT		
Neem	64.64	64.64	57.78	66.67	60.00	62.67[a]	IV
Red chili	36.67	26.67	33.33	48.89	46.67	38.44[b]	II
Garlic	33.33	30.00	30.00	31.11	31.11	31.11[c]	II

HAT = Hours after treatment

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

Table 6. Repellent effect of doses of different plant extracts on *Dermestes* sp. in treated dried fish at different HAT (interaction of dose and time)

Doses (%)	Repellency rate					Mean repellency	Repellency class
	1 HAT	2 HAT	3 HAT	4 HAT	5 HAT		
60	35.56	26.67	31.11	33.33	31.11	31.56^{c}	II
80	46.67	38.89	36.67	51.11	46.67	44.00^{b}	III
100	52.22	55.56	53.33	62.22	60.00	56.67^{a}	III

HAT = Hours after treatment
Within column values followed by different letters are significantly different (P<0.01).

Table 7. Repellent effect of different plant extracts at different dose level on *Dermestes* sp. in treated dried fish at different HAT (interaction of plant, dose and time)

Name of the plants	Doses (%)	Repellency rate					Mean repellency	Repellency class
		1 HAT	2 HAT	3 HAT	4 HAT	5 HAT		
Neem	60	46.67	33.33	33.33	40.00	33.33	37.33^{cd}	II
	80	66.67	73.33	60.00	73.33	66.67	68.00^{b}	IV
	100	80.00	86.67	80.00	86.67	80.00	82.67^{a}	V
Red chili	60	40.00	26.67	40.00	40.00	40.00	37.33^{cd}	II
	80	40.00	20.00	20.00	53.33	46.67	36.00^{cd}	II
	100	30.00	33.33	40.00	53.33	53.33	42.00^{c}	III
Garlic	60	20.00	20.00	20.00	20.00	20.00	20.00^{e}	I
	80	33.33	23.33	30.00	26.67	26.67	28.00^{de}	II
	100	46.67	46.67	40.00	46.67	46.67	45.33^{c}	III

HAT = Hours after treatment
Within column values followed by different letters are significantly different (P<0.01).

CONCLUSION

The LC_{50} values of red chili, neem and garlic were 1.11%, 1.81% and 4.85%, respectively at 24 HAT, which indicated that red chili was the most toxic against adult demisted beetle. Red chili possessed the highest toxic effect at 48 HAT (0.87%) and 72 HAT (0.03%). All of the three plant extracts had repellent action on adult *Dermestes* sp. in dried fish. The highest mean repellency was observed in neem (62.67%) extract followed by red chili (38.44%) and garlic (31.11%). Repellent effects increased proportionally with the concentration of plant extracts. It was thus concluded that treated botanical materials had both repellent and toxic effects against demisted beetles.

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