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.aboratory Evaluation of *Excocearia indica* (Willd.) Muell. Arg Seed Kernel Extract Against Common Filarial Vector, *Culex quinquefasciatus* Say (Diptera:Culicidae) Larvae

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.bstract
ne ethyl acetate extract of *Excoecaria indica* Willd. seed kernel were evaluated for larvicidal activity against common fiarial ector, *Culex quinquefasciatus* Say (Diptera: Culicidae) at different instars under laboratory conditions. The LD_{50} values were 9.36, 17.76, 15.57 and 11.96; 16.28, 13.87, 13.14 and 10.99; 30.23, 28.32, 27.03 and 47.29, 45.71, 43.74 and 6.90 mg/10ml for 1st, 2nd, 3rd and 4th instar larvae *Culex quinquefasciatus* at 3, 6, 12 and 24 hour post exposure espectively. The results also showed that the late instar larvae were more resistant to the extract than those of early stars.

ntroduction
losquito control is one of the major problems of the world oday in view of its vector nature. Various control measures e adopted to control disease-transmitting mosquitoes. articularly, chemical insecticides are commonly used for ontrolling mosquitoes in most parts of the world Schofield, 1993; Pal, 1994). But in recent years one of the ain reasons for the failure of the mosquito control rograms is resistance to these insecticides. Generally isects develop resistance to insecticides due to repeated se of the same insecticides for a long time (Hossain *et al.*, 995). Apart from the development of insecticidal esistance in arthropod vectors of tropical disease, the icreased costs of synthetic chemical insecticides and icreased public concern over environmental pollution ecessitate a continued search for alternative, cheaper ector control methods which require little or no ophisticated technology but give excellent results (Minjas nd Sarda, 1986). In view of this, the study of biologically ctive materials with antilarval properties has attracted onsiderable interest (Kalyanasundaram and Das, 1985; (umar and Dutta, 1987). It is estimated that over 4,00000 ioactive compounds exist but only about 10,000 of them ave been characterized chemically (Swain, 1977). Many lant extracts of terrestrial origin have been reported to uppress mosquito larval populations (Chavan and Nikam, 1982; Saxena and Yadav, 1983) and suggested to be dvantageous for field use in mosquito control programmes (Kalyanasundaram and Das, 1985). *Excoecaria indica* Willd. Family Euphorbiaceae) is an evergreen glabrous tree up to 1m high and found throughout South-east Asia, including angladesh (Hooker, 1885; Prain, 1963; Anon, 1972). The uits of the plant are reported to be poisonous, and causes listers (Kiamuddin *et al.*, 1979). The seed oil from *E. indica* ontains a mixture of three compounds, namely Sapintoxin , Sapintoxin B and 4 α-Sapinine (Miana *et al.*, 1977; vans and Soper, 1978; Taylor *et al.*, 1981a,b). The plant rts are often used as antidote for nailorn, scabies and other skin diseases in rural Bangladesh (Howlader *et al.*, 1992). There are many reports that deal with phytochemical effects in many species of dipteran insects (Chavan *et al.*, 1982; Kalyanasundaram and Das, 1985).

Materials and Methods

Extraction Method: Fresh ripe fruits of *E. indica* were collected from Khulna, Southern Bangladesh. The seeds of the fruits were shade-dried under sunshine for seven days and were pulverized to fine powder using a mortar. The powder was extracted 3 times with 100 per cen ehtyl acetate into a volumetric flux and each round of extraction consisted of powder: liquid (w/v) in the 1:3 ratio. Combined extracts were collected into a conical flask and then filtered by Whatman filter paper (11.0 cm dian). Finally, the solvent was completely evaporated using a vacuum rotary evaporator and the extracted residual materials were defined as the standard extract and it was stored in a refrigerator at 4°C until tested.

Test insects: To ensure a constant supply of the test insects, *C. quinquefasciatus* larvae at different instars used in bioassay were reared in the laboratory at room temperature of 25±5°C and a relative humidity of 70±10°C. Larvae were fed with powdered dry yeast glucose granules (1:3) dissolved in distilled water. Adults were fed with 10% glucose solution soaked in cotton on petridishes. In addition to glucose feeding the adults females in Gerberg mosquito cages (Gerberg, 1970), were also fed with chick blood twice a week regularly.

Larvicidal effects: The extracts were tested for the larvicidal action at different concentration, viz. 0 (control), 5-, 10,- 15-, 20-, 25-, 30-, 35-, 40-, 45- 50- mg/10ml after diluting the stock solutions with water. Two ml of dimethyl sulfoxide were added per mg of extract to make an even solution. The prepared doses were used in 3 replications, each having 60 early 1st, 2nd, 3rd and 4th instar mosquito larvae in test tubes with food medium. The mortality of the larvae at different instar was assessed after 3-, 6-, 12- and 24-hr of treatment. The same number of larvae was kept on untreated medium as controls. Drowning malformed larvae were recorded as being dead. The mortality was determined by counting survivors at the end of exposure period and the control mortality was adjusted by using Abbott's formula (Abbott, 1925) and the results were subjected to probit analysis following the methods in Busvine (1971). The experiments were conducted at 26 ± 2°C.

Results and Discussion

The results of the larval susceptibility of *C. quinquefasciatus* to *E. indica* seed extracts are present in Table 1 and Fig. 1. The extracts were effective against the larvae of different instars. The results showed that the mortality of the larvae increased as the doses of *E. indica* were increased. The same trends were also observed in case of time elapse mortality. It was observed that many larvae failed to ecdyse to perfect pupae, producing larvae-pupal intermediates which were short-lived. The 4th instar larvae of *C. quinquefasciatus* were more resistant to *E. indica* than the 1st instar larvae. The LD_{50} values of the extract were age-dependent. This may clearly support the ideas of others that insect age plays an important role in influencing susceptibility (Kumar and Dutta, 1987; Mwangi and Mukiama, 1988). It would appear that 4th instar larvae are much more resistant to *E.indica* extract compared to other. A comparable observation on delayed lethal effects has been made between *Anopheles stephensi* and *Aedes togoi* when compared to *Aedes aegypti* using neem seed kernel extract and pure azadirachtin (Zebitz, 1986). The sluggish movement and peculiar coiling of treated larva seem to suggest some neural or muscular disturbance b some active principle; which might be cause acute leth effects. This observation more or less similar to Kiamuddi *et. al*,(1979). The delayed lethal effect of the extrac however, is more likely to be caused by a disturbance the endocrine mechanisms that regulate moulting a metamorphosis. This mechanisms of action has bee postulated previously for neem seed kernel extracts (Zebit 1986).

It is concluded that the *E. indica* seed extracts in eth acetate offer a significant potential as new control agen against *C. quinquefasciatus* larvae. However, more wor are to be directed towards this line with differe concentration, extraction and mosquito species.

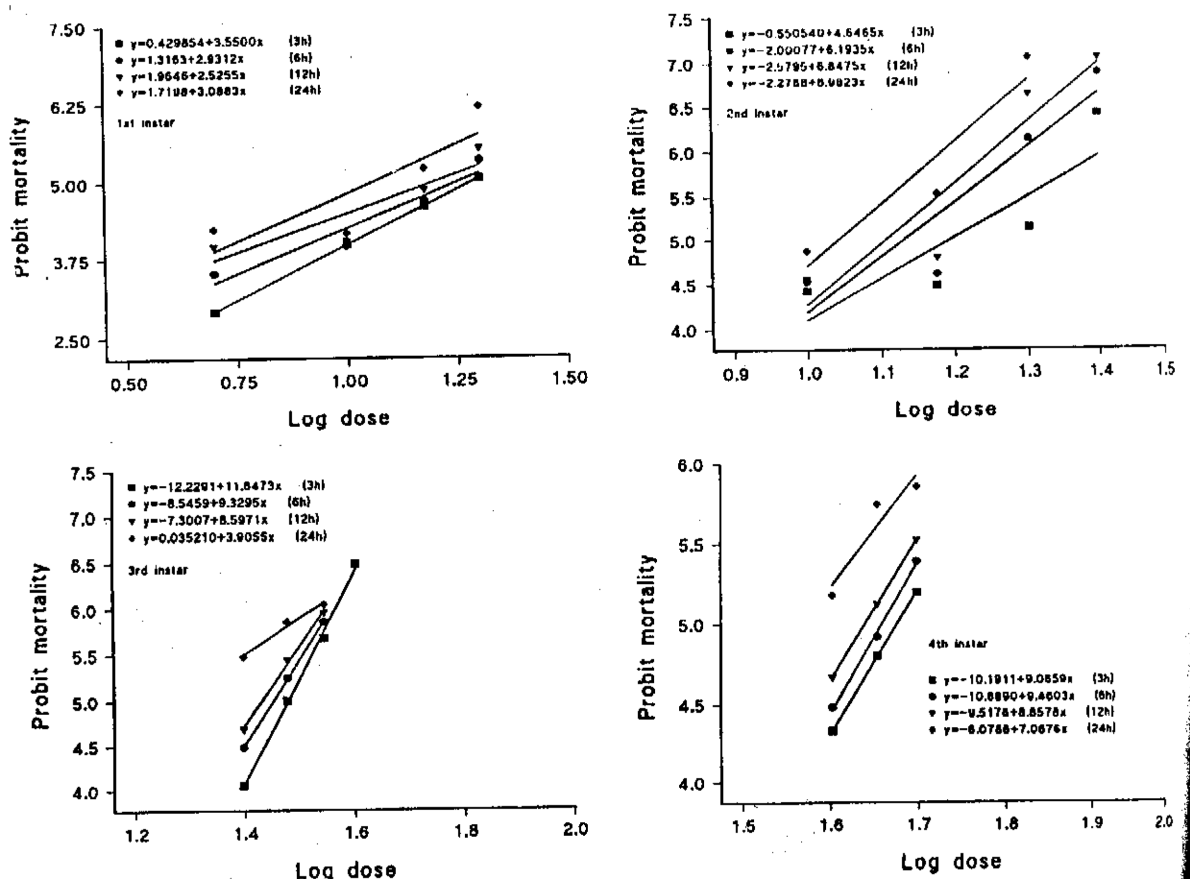

Fig. 1: Probit regression lines for the mortality of different instar larvae of *C. quinquefasciatus* treated with ext Excocearia Indica seed extracted in ethyl acetate.

able 1: Mortality data of *C. quinquefasciatus* larvae at different instars treated with compounds of *E. indica* seed extracted in ethyl acetate.

arval instar	Duration of treatment (hrs.)	LD_{50} value (mg/10ml water)	95% conf. limits lower - upper	X^2 value (df)
	3	19.362	16.47 - 22.76	1.030(2)^{ns}
t	6	17.762	15.13 - 20.85	4.203(2)^{ns}
	12	15.574	10.82 - 22.42	10.067(2)**
	24	11.965	7.80 - 18.35	21.253(2)***
	3	16.285	13.37 - 21.43	16.466(2)***
nd	6	13.870	10.88 - 17.68	18.180(2)***
	12	13.142	10.27 - 16.83	19.564(2)***
	24	10.994	8.34 - 13.96	5.316(1)*
	3	30.226	29.12 - 31.37	0.294(2)^{ns}
rd	6	28.322	26.89 - 29.82	0.169(1)^{ns}
	12	27.036	25.50 - 28.66	2.310(1)^{ns}
	24	18.838	12.49 - 28.40	4.357(1)*
	3	47.292	44.83 - 49.89	0.003(1)^{ns}
th	6	45.708	43.57 - 47.95	3.048(1)^{ns}
	12	43.738	41.54 - 46.04	2.435(1)^{ns}
	24	36.896	31.95 - 42.59	0.673(1)^{ns}

P<0.05; ***P<0.001; ns-not significant.

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