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Lethal Effects of Neem Fruit Extract Against Mosquitoes as Compared to Killifish

M. Farhanullah Khan[1], M. Atiqullah Khan[2] and Rahila Tabassum[3]
[1]Pestic Research Lab., PARC, University Karachi, Karachi, [2]Marine Reference Collection and Resource Centre, University of Karachi, Karachi [3]Department of Zoology, University of Karachi, Karachi-75270, Pakistan

Abstract: Toxicity of neem extract (RB-a) against *Anopheles stephensi* (Mosquito) and *Aphanius dispar* (Killifish) was studied. The applied concentration of RB-a against *Aphanius dispar* were 20,30,40,50 and 60 ppm, while the mortality percent was 6.67, 36,67,53.33,80 and 90% respectively. The LC_{50} was found to be 36.37 ppm and LC_{90} was found to be 61.04 ppm. The applied concentrations of RB-a were 30,60,90,120 and 150 ppm against *Anopheles stephensi,* the mortality percent were observed as 15,40,60.80 and 85% respectively. The LC_{50} against this pest was found to be 69.4 ppm and LC_{90}, was found to be 182.77 ppm.

Key words: Neem extract, Toxicity, *Anopheles staphensi, Aphanius dispar*

Introduction

The ever increasing cost and ecological problems posed by the pesticides has down the attentions of researchers to seek better, cheaper and least hazardous pesticides. Virtually natural pesticides qualify the standard to some extend, specially the neem is most promising one. Neem *(Azadifachta indica)* has been studied to control agricultural pest and domestic as well Rukhsana *et al.* (1993). In a common publication of WAO, FAO< IRRI and UNEP Smith and Cervantes (1988) have emphasised on conducting vector control studies with reference to neem. Keeping in view that usually a part of pesticides always goes into water and with a view of mosquitoes ecology. The present study was conducted on toxicity of neem fraction on mosquito larvae and toxic effects of neem on a fish *Aphanius dispar. Aphanius dispar* is found near the coast around mangroove area and also found in fresh water as well as esturine. This species is also an important organism of aquatic food web (Khan *et al.,* 1994). Therefore, *A. dispar* was selected as a good representative of non-target aquatic species to study pesticide toxicity.

Materials and Methods

RB-a ethanolic crude extract of neem seed kernel was provided during the studies i.e, 1998-99 by HEJ Institute of Chemistry, University of Karachi. Five different concentration were selected after preliminary trails. The selected concentration for *Anopheles stephensi* (Mosquitoes) and five concentration for *Aphanius dispar* (Killifish). The experimental insect *Anopheles stephensi* was kept in insectory of Department of Zoology, while marine fish *Aphanius dispar* were collected from the coastal Area of Karachi region. After collection they were kept in aquarium in under laboratory conditions, Fish of similar size and age were selected for toxicity determination. While 3rd instar larvae of mosquito were used for toxicity determination. Toxicity against mosquito was determined with WHO method as described by Naqvi *et al.* (1994). Fish toxicity was determined as containers of five litre capacity were filled with 3 litre of Marine water and neem extract was add to make the desired concentrations. Toxicity count was made after 24- hours of treatment. Experiment was repeated three times and always in triplicate sets along with control for both organisms i.e mosquito and killifish, The data was analyzed with probit analysis (Fig. 1, Table 1 and 2).

Results and Discussion

Toxic effect of RB-a (Crude neem seed kernel extract) on 3rd instar mosquito larvae (*Anopheles stephensi*) and marine fish (*Aphanius dispar*) were studied as shown in Table 1 and 2. The selected concentration of RB-a against mosquito larvae were 30,60,90,120 and 150 ppm while mortality percent at these concentration were 15,40,60.80 and 85% respectively. The LC_{50} was found to be 68.00 ppm and LC_{90} was found to be 182.77 ppm. The applied concentration against killifish were 20, 30, 40, 50 and 60 ppm. While the mortality percent was 6.67,36.67,53.33,80 and 90% respectively. Whereas LC_{50} was found to be 36.37 ppm and LC_{90} was found to be 61.04 ppm. *Anopheles stephensi* and *Aphanius dispar* were tested laboratory conditions. Both organisms belong to the aquatic environment. Pesticides when used in agriculture directly or indirectly produce pollution in aquatic environment and this contamination is harmful for aquatic fauna and flora. In present study the effect of natural product RB-a was studied against mosquito larvae and marine fish. In mosquito Larvae LC_{50} was found to be 68 ppm. Whereas in the case of Killifish mortality was observed 90% at 60 ppm while 6.67% mortality at 20 ppm was found, The LC_{50} was found to he 36.37 ppm. The neem extracts were used against different organisms and found effective. Naqvi *et al*. (1989) compared ne'ern products (RB-a, RB-A with malathion as controlling agent for white, flies on brinjal crop and found better results of neem products, Nurulain *et al.* (1989) reported the toxicity of FIB-a, RB-A, Margosan-D™ and malathion against dusky cotton bug in laboratory conditions. They found malathion more effective than neem compounds. Naqvi *et al*. (1992) reported RB-a effective against grasshopper in field and laboratory condition. In field condition 15% extract was shown 58.02% mortality. Naqvi *et al.* (1993) reported in laboratory condition the LC_{50} was found to be 70.71 uglcm^2 while in field trails after 24 hours of treatment 12.36% population was found. Khan *et al.* (1995) observed effect of RB-a. RB-b sod cypermethrin against 3rd instar larvae of *Aedes aegyptic* of different locations and reported that RB-a was more toxic than FIB-b and cypermethrin was found most effective in all localities. In present study RB-a showed some effects against mosquito larvae i.e. 150 ppm dose exerted 85% mortality. While LC_{50} value was 68 ppm oli these results are in agreement with the previous authors. RB-a was found more effective against *Aphanius dispar* as at 60 ppm doss 90% mortality was observed against this species. Jahan *et al*. (1994) studied comparative toxic effect of deltamethrin and SDS (deem formulation) against *Barbus punctius.* They reported neem formulation useful for pest management. While in present study AB-a was found more toxic against *Aphanius dispar* (nontarget) than *Anopheles stephensi* (Pest sp.).

The studies were conducted with the concept that neem is eithe least harmful or less to fish than pest spp. (Margraf 1988) however, contrary to the general concept that neem is almost not lethal to fish fauna, the under test fish was found more susceptible than mosquito. Jayaraj (1992) observed no mortality in *Oreachretnis*

Table 1: Toxicity of RB-a by WHO Method against *Anopheles stephensi*

Dose in ppm	Mosquitoes exposed	Average mortality	Mortality %	Probit mortality
control	20			
30	20	3	15	3.1
60	20	8	40	3.6
90	20	12	60	3.8
120	20	16	80	4.0
150	20	17	85	4.0
Regression equations for *A. stephensi*		Y = 4.174033 + 3.04557 [X - (2.39877811		x^2 = 1.63
Toxicity and Range at 95%	LC_{50} = 68.00 ppm	--- 63.1	--- 40.04	
	LC_{90} = 182.77 ppm	--- 153.6	--- 217.47	

Table 2: Toxicity of RB-a against *Aphanius dispar*

Dose in ppm	Fish exposed	Mortality	Mortalty %	Probit mortality
Control	30	0	-	-
20	30	2	6.67	2.9
30	30	11	36.67	3.77
40	30	16	53.33	4.00
50	30	24	80.00	4.20
60	30	27	90.00	4.39
Regression equations for *Aphanius dispar*		Y = 5.9284 + 5.535 [X - (0.5478)]		X^2 0.7828
Toxicity and Range at 95%	LC_{50} = 36.37 ppm	--- 32.7	--- 40.04	
	LC_{90} = 61.04 ppm	--- 49.46	--- 77.57	

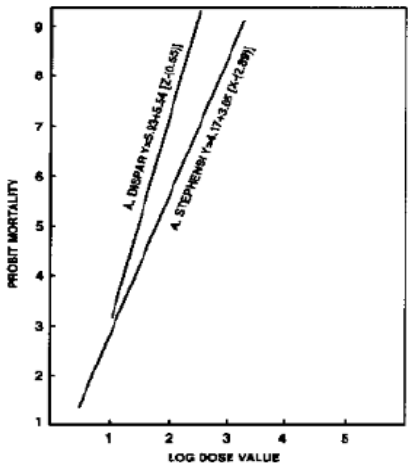

Fig. 1: Comparative toxicity of rba against *Anopheles stephensi* and *Aphanius dispar*

nifaticus after application of neem oil 50% EC at -0.01%, Zebitz (1987) and Fernandez *et al*. (1992) reported neem extracts produced toxic effect on fish fauna in present studies. It was found that neem extract AB-a is toxic to fish than mosquitoes. Therefore, on the basis of these studies it could be suggested that in ogre-mosquito ecosystem neem or at least RB-a based pesticide should he used carefully with assurance that surrounding spp. are not endangered with it.

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