Annals of Arid Zone 41(2): 169-173, 2002

Toxicological Evaluation of Some Organophosphorus Compounds against Larvae of *Culex quinquefasciatus* in Bikaner District, Rajasthan

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Abstract: Effects of six organophosphorus compounds, viz., malathion, fenitrothion, fenthion, dichlorvos, temephos and chlorpyrifos were evaluated on larvae of *Culex quinquefasciatus*, the vector of lymphatic filariasis. Different diagnostic concentrations supplied by WHO were used and per cent mortalities determined. LC_{50} values, as determined by probit analysis, were 1.273, 0.1091, 0.0947, 0.0458, 0.0076 and 0.0038 mg L^{-1}, respectively. Chlorpyrifos and temephos were the most toxic where 100% mortality was observed while fenitrothion, fenthion and dichlorvos were found in between these two extremes. Regression coefficient (slope), fiducial limits to LC_{50} and LC_{95} and heterogeneity of the response (x^2) were calculated for each insecticide.

Key words: *Cx. quinquefasciatus*, organophosphate larvicides, desert.

Anti-larval operations with organophosphate (OP) compounds like temephos and fenthion constitute one of the major activities for controlling the vector breeding in urban areas under the National Filaria Control Program (NFCP). About 15 mosquito species, belonging to both anopheline and culicine, have been reported to breed in different habitats of all the four tehsils of district Bikaner (Bansal, 1998) in different seasons, but *Cx. quinquefasciatus*, a cosmopolitan and nuisance vector of lymphatic filariasis, caused by *Wuchereria bancrofti*, breeds throughout the year (Bansal *et al*., 1994). It breeds profusely in construction pits, muddy wood covered shallow wells used for storing water and large water bodies in low lying areas, contaminated with organic waste.

Although several studies have been done on the insecticide susceptibility of the larvae of this species in several parts of India (Joshi *et al*., 1979; Krishana Rao *et al*., 1989; Thomas *et al*., 1991; Ganguly *et al*., 1994), no study has been done so far on the larvae in this part of the Thar Desert. Therefore, studies on the susceptibility of larvae of this species to different insecticides in this area is an essential pre-requisite for effective vector control, which would help in selecting the most toxic insecticide and for evaluating possible future resistance status.

Materials and Methods

Fully fed, adult female mosquitoes were collected early in the morning from the human dwellings and cattle sheds in different parts of Bikaner, Loonkaransar, Nokha and Kolayat tehsils. The mosquitoes were collected using an aspirator, and kept in Barraud cages provided with cotton pads, soaked in 10% glucose solution. An enamel tray with water was kept inside the cage for laying egg rafts. The immature 1st to late 3rd or early 4th instar larvae were reared in the laboratory and used for the tests. During this period the larvae were fed on finely powdered dog biscuits and yeast powder in the ratio of 3:1.

To determine larval susceptibility, standard method as recommended by WHO, was used (WHO, 1981). Standard concentrations of OP larvicides were obtained from NAMP (Delhi) and different manufacturing firms in India. Stock solutions of technical grade samples were prepared in absolute alcohol and preliminary tests were done with a wide range of dilutions. Various test concentrations were prepared as per requirement by adding 1 mL of the standard insecticide solution to 249 mL of tap water. Control tests were conducted by adding 1 mL of the solvent to 249 mL of water. To each of the beaker containing different test concentrations, 25 healthy late 3rd or early 4th instar larvae were released after straining water through a minnow net. Per cent mortalities were calculated 24 hours later by counting both dead and moribund larvae. Larvae were considered moribund if they failed to flex head to siphon when provoked with a glass rod. All tests were carried out at a temperature of 28±2°C and RH 75±5%. All the treatments were repeated four times to investigate variations and the average was taken. The data were corrected by using Abbott' formula (Abbott, 1925) when mortality was below 20% in control experiments. The LC_{50} and LC_{95} values were computed using probit regression analysis (Finney, 1971).

Results and Discussion

The results of larval susceptibility to all the six OP insecticides are given in Tables 1 and 2. From the tests it was clear that chlorpyrifos and temephos were the most toxic among the insecticides tested against larvae of *Cx. quinquefasciatus*, the LC_{50} being 0.0038 and 0.0076 mg L^{-1}, respectively, and a 100% kill at the standard diagnostic concentration. A 100% kill with temephos was also observed in the field-collected and laboratory populations of this species at Panaji, Goa (Thavaselvam *et al.*, 1993). Studies carried out in other parts of India also revealed that temephos was a very toxic larvicide, the LC_{50} being about 0.0015 in Pune (Ganguly *et al.*, 1994), 0.0017 (Thomas *et al.*, 1991) and 0.0022 (Mittal *et al.*, 1994) in Delhi and 0.0076 mg L^{-1} in our studies at Bikaner. However, tests carried out at Rajahmundary town (AP) showed that temephos 50% EC was not much effective in drastically reducing the larval and pupal density, even at dosages four times higher than the recommended one (Patnaik *et al.*, 1997). Tests carried out with chlorpyrifos showed that it was the most toxic (LC_{50}-0.0038 mg L^{-1}) among the insecticides tested in the present investigation. Studies at Gwalior (MP) (Gopalan *et al.*, 1996) also showed it to be about 100 times more toxic than malathion, the LC_{50} in susceptible population being 0.0003 and 0.03 mg L^{-1}, respectively, but continuous exposure of the larvae to malathion upto 25th generation raised the LC_{50} to 2000 times from 0.03 to 61.09 mg L^{-1} malathion, however, in the present study it was found least toxic (LC_{50}-1.273 mg L^{-1}), suggesting that larvae of this species developed a high degree of resistance towards malathion in this desert area. Tests carried out with fenitrothion, fenthion and dichlorvos showed that larvae of *Cx. quinquefasciatus* were also resistant to these insecticides

Table 1. Susceptibility status of larvae of Cx. quinquefasciatus to six organophosphorus larvicides in district Bikaner

Insecticide	Concentration	% Experimental mortality	% Corrected mortality
Malathion	Control	3.0	-
	0.125	12.0	12.0
	0.625	34.0	34.0
	1.250	44.0	44.0
	2.500	64.0	64.0
	3.125	82.0	82.0
Fenitrothion	Control	6.0	-
	0.025	12.0	6.4
	0.050	29.0	24.5
	0.100	42.0	38.3
	0.125	66.0	63.8
	0.250	85.0	84.0
Fenthion	Control	5.0	-
	0.025	12.0	7.4
	0.050	27.0	23.2
	0.100	58.0	55.8
	0.125	69.0	67.4
	0.250	79.0	77.9
Dichlorvos	Control	6.0	-
	0.010	13.0	7.4
	0.025	32.0	27.7
	0.050	52.0	48.9
	0.100	80.0	78.7
	0.125	92.0	91.5
Temephos	Control	4.0	-
	0.0025	10.0	10.0
	0.0050	18.0	18.0
	0.0100	82.0	82.0
	0.0250	97.0	97.0
	0.0500	100.0	100.0
Chlorpyrifos	Control	6.0	-
	0.0010	14.0	8.5
	0.0025	24.0	19.1
	0.0050	76.0	74.4
	0.0100	98.0	97.9
	0.0250	100.0	100.0

because a 100% mortality was not observed at the standard concentrations. The observed LC_{50} values were 0.1091, 0.0947 and 0.0458 mg^{-1}, respectively. These values were quite high when compared with results obtained with the larvae of *Cx. quinquefasciatus* in other parts of India (Thomas *et al.*, 1991; Ganguly *et al.*, 1994; Mittal *et al.*, 1994; Gopalan *et al.*, 1996) with the same insecticides. Adults of *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* were also found resistant

Table 2. Probit regression line analysis of the mortality data of larvae of Cx. quinquefasciatus

Insecticide	Regression coefficient (slope)	Regression equation	Intercept	Chi-square(x^2)/ Heterogeneity (DF)	LC_{50}±S.E. (Fiducial limits)	LC_{95}±S.E. (Fiducial limits)
Malathion	1.24	1.24x+2.39	2.39	2.02(3)	1.2730±0.0124 (0.8380-1.9340)	26.7500±0.0215 (5.9530-120.30)
Fenitrothion	2.39	2.39x+0.23	0.23	2.46(3)	0.1091±0.0011 (0.0863-0.1378)	0.5294±0.0014 (0.2813-0.9965)
Fenthion	2.53	2.53x+0.00	0.00	1.01(3)	0.0947±0.0010 (0.0769-0.1117)	0.4212±0.0012 (0.3082-0.5755)
Dichlorvos	2.30	2.30x+1.18	1.18	0.80(3)	0.0458±0.0011 (0.0356-0.0589)	0.2365±0.0014 (0.1219-0.4590)
Temephos	3.53	3.53x-1.64	-1.64	5.43(3)	0.0076±0.0001 (0.0060-0.0097)	0.0222±0.0001 (0.0117-0.0421)
Chlorpyrifos	3.47	3.47x-0.49	-0.49	6.33(3)	0.0038±0.0001 (0.0030-0.0048)	0.0113±0.0001 (0.0061-0.0213)

All values of LC_{50} and LC_{95}, along with their fiducial limits, are in mg^{-1}.

to a certain degree when exposed to the insecticide-impregnated papers of DDT, dieldrin, malathion, fenitrothion and propoxur, but susceptible to permethrin, a synthetic pyrethroid in Bikaner (Bansal and Singh, 1995a,b). These results clearly indicate that larvae have developed resistance towards many of the organophosphate compounds tested in the present study. These results suggest that the resistance is slowly building up in the population in this area and its level has grown many times. High resistance might be due to the strain variations and their adaptability to the harsh desert climatic conditions, thus making them fit not only to pressures of natural selections, but also to many insecticides as well.

Acknowledgements

Authors are thankful to NAMP (Delhi) for providing the WHO standard samples of insecticides and different manufacturing firms in India for supplying the technical grade samples.

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