

COMPARATIVE TOXICITIES OF FOUR WHO-RECOMMENDED LARVICIDES AGAINST LAB STRAIN OF *ANOPHELES STEPHENSI* IN IRAN, 1999

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Abstract

Investigation on the current response of *An.stephensi* larvae to four WHO recommended larvicides viz. malathion, temephos, chlorpyrifos and fenitrothion, were carried out in the laboratory in 1999. Diagnostic concentrations of pesticides only yielded 100% mortality with malathion. In contrast, levels of susceptibility to temephos, chlorpyrifos and fenitrothion were lower. Fenitrothion (0.125 mg/l), chlorpyrifos (0.025 mg/l) and temephos (0.625 mg/l) killed 72%, 90% and 87% of the population of *An.stephensi*, respectively. At the LC₅₀ level the efficacies of chlorpyrifos and fenitrothion was higher than malathion and temephos. Relative toxicity of chlorpyrifos and fenitrothion was 6 and 24 time more than temephos and malathion. The findings of this study suggests that the diagnostic dose of organophosphate larvicides depend on time, location, strain and genetical background of resistance to insecticides, hence they can not be attributed to all species of *Anopheles*.

Introduction

Malaria has always been considered as the most important vector-borne disease in Iran due to its effect on the socio-economy of the populations. WHO recommends

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that malaria control receives of multilateral political support at different levels, is inserted into the health system of country, that there is an efficient community contribution and a necessary resource supplement (budget and expert staff). Our plan for control of malaria is in line with WHO recommendations. At the national malaria control strategies selective vector control against adult and larval stages is an attempt to reduce the man-vector contact and transmission of parasites to human.

Eight species of Anopheles including *An.stephensi*, *An.culicifacies*, *An.dthali*, *An.superpictus*, *An.maculipennis*, *An. pulcherrimus*, *An.sacharovi* and *An.fluviatilis* play important roles in malaria transmission in different parts of Iran. Among which *Anopheles stephensi* is known to be an important malaria vector in the Persian Gulf, the Middle-East and Indian subcontinent. As a result of the continuous application of insecticides in these regions, *An.stephensi* populations are known to be resistant to DDT (2,3,14), dieldrin (4,21) and malathion (6,7,8,9,11,12,13,18). There are as well some reports of pyrethroid resistance in *An.stephensi* based on laboratory selection (1,10,15,17). In the absence of any previous report on insecticide susceptibility of *An.stephensi* larvae in Iran, It was thought necessary to determine susceptibility in this species to WHO-recommended larvicides; malathion, chlorpyrifos, fenitrothion, and temephos.

Materials and methods

Larvae were tested with different concentrations of insecticides at the early 4th instar in a room with a temperature of 25 ± 1 °C according to the WHO method (20). Mortality counts were made after 24 hour exposure to the insecticide. In the analysis both dead and moribund larvae were considered as dead, and the alive larvae were scored separately. Only larvae of uniform size were considered during all of the tests. At each concentration 200 mosquitoes representing individuals of 25 larvae were used at four interval occasions. Parallel control tests were done by adding 1 ml alcohol without insecticide in 249 ml of water. The percentage mortality was corrected with control using Abbotts' formula. Dosage mortality regression lines were determined by the probit analysis method (5), using the probit 79 programme on a computer. Goodness of fit of the points to a straight line were tested by Chi-square

analysis. A laboratory strain of *An.stephensi* named Tehran that is resistant to both DDT and dieldrin at the adult stage and susceptible to all insecticides at the larval stage were used. This strain has been reared in our insectary for 400 generations. Different concentrations of alcoholic solutions of insecticides which is supplied by WHO in 50 ml bottles were used. Standard stock solutions of insecticides were as follows:

malathion 781.25, 156.25, 31.25, 6.25 mg/l; temephos 156.25, 31.25, 6.25, 1.25 mg/l; fenitrothion 31.25, 6.25, 1.25, 0.25 mg/l and chlorpyrifos 6.25, 1.25, 0.25, 0.05 mg/l.

In some cases lower logarithmic concentration of insecticides were diluted by adding required volume of alcohol solvent to the insecticide.

Results and discussion

The results of the studies on the efficacy of four organophosphorus larvicides; malathion, chlorpyrifos, temephos and fenitrothion against larvae of *An.stephensi* are shown in Fig. 1. Malathion at the diagnostic dose (3.125 mg/l) yielded 100% mortality in the population. Lower mortality was obtained when they were tested with fenitrothion, chlorpyrifos and temephos. Diagnostic dose of fenitrothion (0.125 mg/l) caused 72% mortality. For chlorpyrifos (0.025 mg/l) and temephos (0.625 mg/l) the figures were 90% and 87%, respectively. In decreasing order of mortality at the diagnostic levels, fenitrothion was the least effective and malathion was the most toxic to the larvae of *An.stephensi*. Comparative toxicities of larvicides are shown in Fig. 2. The LC_{50} and LC_{90} is used to assess the relative toxicities of pesticides. In order to find correlation between mortality and concentration probit-regression line parameters were calculated (5). The results are presented in Table 1 and Fig.3. At the LC_{50} level fenitrothion exhibited lowest value (0.00 mg/l). The same figure can be seen by chlorpyrifos. A linear correlation was found between the response of *An.stephensi* and the doses of insecticides. The regression lines of different insecticides are plotted in Fig.3. Results of X^2 test showed the response of *An.stephensi* to different insecticides statistically significant values.

indicating that proportions of the species differed in response and the response was not homogenous. In a study diagnostic doses of malathion, fenitrothion and temephos caused 61%, 84% and 100% mortality, respectively in the population of lab strain of *An.stephensi* in India (19), with high resistance to DDT, fenitrothion and malathion but were susceptible to temephos.

In view of these results, it can be concluded that diagnostic dose is flexible, and may vary in time and space. The effective management of resistance monitoring depends on understanding the genetical, biochemical and behavioural factors determining resistance in the mosquitoes.

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Table 1- Probit regression line parameters of *An.stephensi* to different larvicides

Insecticide	a	B±SE	LC ₅₀ ±95% C.I	LC ₉₀ ±95% C.I	X ² _(df)	P
Malathion	1.37	1.03±0.16	0.016	0.240	46.2 (1)	<0.0001
			0.047	0.810		
			0.136	10.57*		
Temephos	0.98	0.507±0.04	0.007	1.730	6.63 (1)	<0.001
			0.012	3.930		
			0.017	12.11*		
Chlorpyrifos	1.90	0.073±0.17	0.0005	0.014	37.03 (1)	<0.0001
			0.0020	0.142		
			0.0620	0.290		
Fenitrothion	1.25	0.47±0.11	0.0002	0.070	37.9 (1)	<0.0001
			0.0020	1.170		
			0.0150	3.710		

* not valid

a= intercept

B±SE= slope ± standard error

LC₅₀±95% C.I= lethal concentration cause 50% mortality in population ± 95% confidence interval

LC₉₀±95% C.I= lethal concentration cause 90% mortality in population ± 95% confidence interval

X²_(df)= heterogeneity about the regression line with degree of freedom

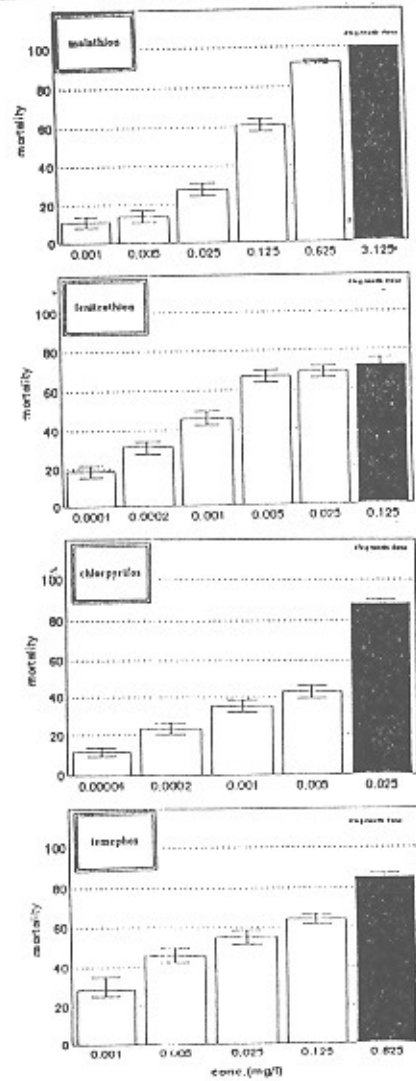


Fig. 1- The mortality of *An.stephensi* to different concentrations of larvicides

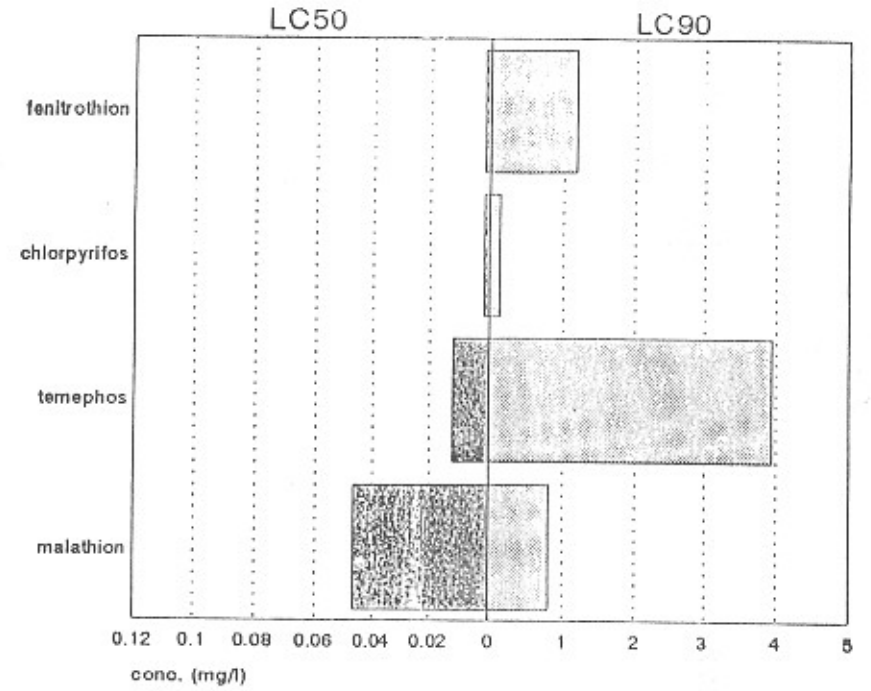


Fig. 2-Comparative toxicities of different larvicides against *An.stephensi* larvae

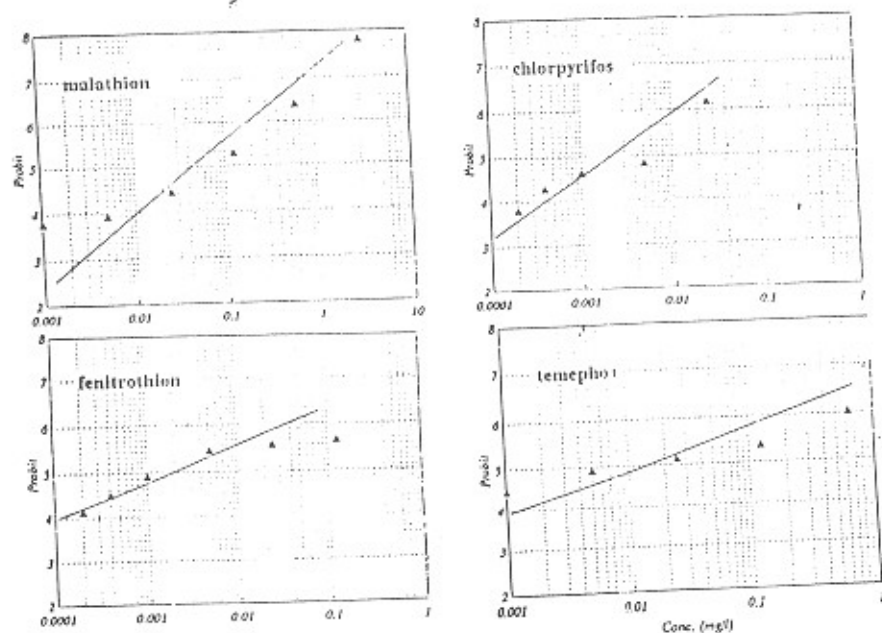


Fig. 3- Probit regression lines of *An.stephensi* to different larvicides

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