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Evaluation of pirimiphos-methyl (50% EC) against the immatures of *Anopheles stephensi/An. culicifacies* (malaria vectors) and *Culex quinquefasciatus* (vector of bancroftian filariasis)

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Background & objectives : In India, temephos and fenthion are used as larvicides in fresh and polluted waters. Since use of same insecticide may precipitate resistant, as an alternative bioefficacy of pirimiphos-methyl—an organophosphorus insecticide was evaluated against immatures of *Anopheles* and *Culex* species in different breeding habitats in District Ghaziabad (U.P.) and Goa.

Methods : Laboratory bioassays were carried out using standard WHO procedure. LC_{50} and LC_{90} were calculated using Probit analysis. Abbott's formula was used to calculate per cent corrected mortality in laboratory exposures while, Mulla's formula was applied for calculating per cent reduction in mosquito immatures under field conditions.

Results : Pirimiphos-methyl was found to be most effective against larvae of *An. stephensi* followed by *An. culicifacies* and *Cx. quinquefasciatus*. The LC_{50} and LC_{90} values against three species were 0.023, 0.032 and 0.04 ppm; and 0.045, 0.057 and 0.114 ppm respectively. In field, pirimiphos-methyl @ 200 g a.i./ha against *Anopheles* species and @ 300 g a.i./ha against *Culex* species can produce > 80 % reduction in density of immatures up to one week in different habitats. Pirimiphos-methyl < 0.25 ppm is not toxic to fish.

Interpretation & conclusion : Pirimiphos-methyl is more effective against anophelines in clean water than culicines in polluted water. This larvicide should not be used at a concentration above 0.25 ppm in habitats harbouring the larvivorous fish.

Key words *An. culicifacies – An. stephensi – Cx. quinquefasciatus* – efficacy – larvicide – pirimiphos-methyl

Larval control during the past had been dependent mainly on the use of chemicals such as Paris green and larvicidal oils. At present organophosphorus insecticides such as temephos and fenthion are used as larvicides in fresh and polluted water respectively, under urban malaria scheme (UMS) in India. Use of the same larvicide for a long-time may, however, precipitate resistance in mosquito larvae[1]. Therefore, it is necessary that some alternate chemicals or other larvicides should also be evaluated for future use. As an alternative to chemical, larvicidal preparations consisting of *Bacillus thuringiensis* and *Bacillus sphaericus* have been evaluated extensively[2–6]. Though, environmental friendly, most of these biolarvicides are not a cost-effective alternative to chemical larvicides. Pirimiphos-methyl, an organophosphorus insecticide, soluble in most organic solvents is a promising agent and

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has been included in the list of World Health Organization (WHO) as a suitable mosquito larvicide[7]. The toxicity of this insecticide is low and the oral and dermal LD_{50} (white rats) values are 2050 and 2000 mg/kg respectively. Only limited trials were carried out with this insecticide in Indian conditions. National Anti Malaria Programme (now National Vector Borne Diseases Control Programme), therefore, also suggested that this new insecticide may be tested for its bioefficacy as a larvicide against different mosquito species under Indian conditions. To rationalise its use in control programme against mosquito vectors, it was considered desirable to evaluate its bioefficacy against vector species of mosquitoes such as *An. culicifacies* (a principal vector of rural malaria), *An. stephensi* (an urban malaria vector), *Culex quinquefasciatus* (vector of filariasis) and also against nontarget mosquito larvivorous fish (*Gambusia affinis*).

Material & Methods

Laboratory bioassays : To determine the laboratory bioefficacy of pirimiphos-methyl against larvae, bioassays were performed as per WHO recommended procedure[8]. Bioassays were carried out at a temperature of $28 \pm 2^{o}C$ and relative humidity 70–80%, using laboratory reared immatures of different species of mosquitoes (late III or early IV instar larvae) were used in batches of 25 larvae in 250 ml water taken in 500 ml beakers. Initially 1% stock solution for the formulation was prepared and later serial dilutions were made with distilled water. Four replicates for each concentration were tested along with concurrent control using only distilled water. Mortality of larvae was recorded after 24 h of exposure. Corrected per cent mortality was calculated by using Abbott's formula which is given below. LC_{50} and LC_{90} values were calculated as described by Finney [9]. The dosage for field-testing was determined on the basis of these bioassays. A dosage equivalent to two or more fold value of the LC_{90} was used in field tests.

$$\text{Corrected \% mortality} = \frac{[\text{\% mortality (test)} - \text{\% mortality (control)}]}{[100 - \text{\% mortality (control)}]} \times 100$$

Toxicity to larvivorous fish : In addition to the bioefficacy against the disease vectors, pirimiphos-methyl was also tested for its safety against the larvivorous fish, *Gambusia affinis*[10]. This fish is being introduced in a number of large water bodies in the rural and urban areas under the Enhanced Malaria Control Project (EMCP). Twenty-five larvivorous fishes (25 to 30 mm in length) were placed in an enamel basin tray containing two litres of stored tap water. Four replicates were used for each concentration. Thus minimum of 100 fishes were tested for each concentration. A pair of control was run concurrently to check the control mortality. Different concentrations of pirimiphos-methyl were used to test the toxicity. The mortality was recorded at 24, 48 and 72 h.

Field evaluation : Field trials were carried out from August to December 2003 in District Ghaziabad (U.P.) and Delhi in pools, wells, ponds, drains, paddy fields and cement tanks against immatures of *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus* and in Goa in cement tanks and waste commodes against *An. stephensi*. The 50% EC formulation was used at the precalculated doses after appropriate dilutions for determining the bioefficacy in field conditions. Concentration of pirimiphos-methyl was made after dilution with water and sprayed with the help of compression pump at the precalculated dosage rate on the water surface area basis in pools, wells, ponds, paddy fields, drains and cement tanks. Before spraying the larvicide average depth of the breeding site was also recorded and taken into consideration for the calculation of the dose to be achieved. The larval density in untreated and treated habitats was determined by dipping method and estimated as density of larvae per dip. The per cent reduction in the post-treatment larval density was calculated using Mulla's formula[11], which is as follows.

$$\text{\% Reduction} = 100 - \frac{C_1}{T_1} \times \frac{T_2}{C_2} \times 100$$

Where, C_1 and T_1 are the pre-treatment densities and C_2 and T_2 are post-treatment densities of larvae per dip in the control and treated habitat respectively.

Results & Discussion

Results of laboratory evaluation of pirimiphos-methyl 50% EC against the larvae of *An. culicifacies*, *An. stephensi* and *Cx. quinquefasciatus* are shown in Table 1. The larvicide formulation was relatively more effective against immatures of *Anopheles* than *Culex* species. The LC_{50} and LC_{90} values against the larvae of *An. culicifacies* and *An. stephensi* were 0.032 and 0.05 ppm; and 0.023 and 0.045 ppm, respectively, as against 0.04 and 0.114 ppm for *Cx. quinquefasciatus.* Almost similar values of the lethal concentrations of pirimiphos-methyl against larvae of *An. culicifacies* and *An. stephensi* have been reported earlier by other workers[12]. It is clear from these observations that concentration of at least 0.1 and 0.2 ppm (2 x LC_{90}) of pirimiphos-methyl will be required for field application against immatures of *Anopheles* and *Culex* spp.

Field trials were carried out in muddy pits and pools, irrigation channels, unused wells, cement tanks, waste commodes, polluted drains and paddy fields to determine the optimum dosage against immatures of *Anopheles* and *Culex* spp. Application of pirimiphos-methyl at the dosage of 100 g a.i./ha and above resulted in 100% reduction in the larval density of anophelines within 24 h after the treatment (Fig. 1).

Table 1. Lethal concentrations (LC_{50} and LC_{90}) of pirimiphos-methyl against mosquito larvae under laboratory conditions

Mosquito species	Lethal concentration (a.i.)/ppm		χ^2 (Degree of freedom)
	LC_{50} (95% confidence limit)	LC_{90}	
An. culicifacies	0.0321 (0.0275 – 0.0374)	0.057	1.79 (2)
An. stephensi	0.023 (0.0192 – 0.0274)	0.045	1.22 (2)
Cx. quinquefasciatus	0.040 (0.0355 – 0.045)	0.114	10.54 (2)

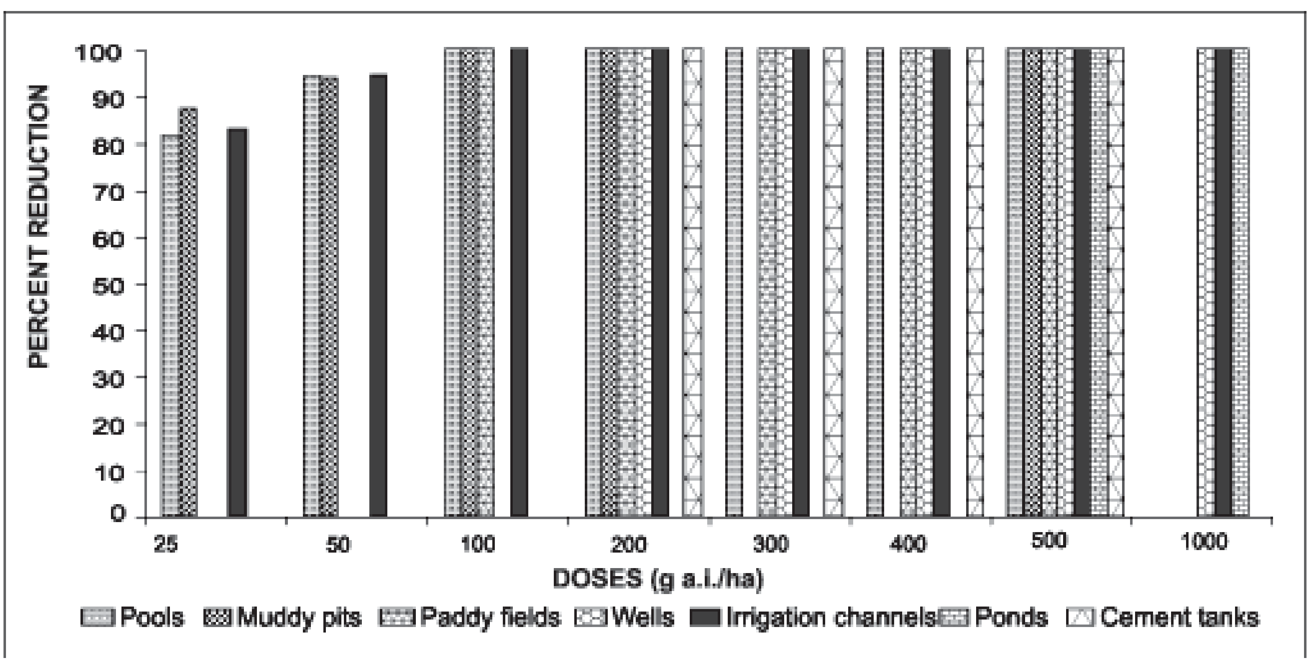

Fig. 1: Evaluation of pirimiphos-methyl 50% EC against immatures of *Anopheles* spp under field conditions in Delhi

Table 2. Bioefficacy of pirimiphos-methyl 50% EC against anopheline immatures at different doses

Study sites	Habitats	% Reduction on Day 7						
		50 g a.i./ha	100 g a.i./ha	200 g a.i./ha	300 g a.i./ha	400 g a.i./ha	500 g a.i./ha	1000 g a.i./ha
Ghaziabad	Pools	51.2	76.3	83.4	100	–	–	–
	Muddy pits	45.1	87.6	100	–	–	–	–
	Paddy fields	–	74	88.2	100	–	–	–
	Unused wells	–	-	88	93	96.5	96	100
	Irrigation channels	36	68.4	92.3	100	–	–	–
Delhi	Cement tanks with vegetation	–	73.3	100	–	–	–	–
Goa	Cement tanks with clear water	–	100	100	–	–	–	–
	Waste commodes	–	80	100	–	–	–	–

Note : Surface area : Pools (8 to 15 m^2); Muddy pits (3.6 to 4.2 m^2), Paddy fields (542 to 916.6 m^2); Wells (2.4 to 3.6 m^2).

However, the bioefficacy (% reduction in the larval density on Day 7 after the treatment) of pirimiphos-methyl against immatures of anopheline species in different types of breeding habitats ranged between 68.4 and 100% @ 100 g a.i./ha and > 80% @ 200 g a.i./ha (Table 2). In pits and pools with *Anopheles* breeding consisting of mainly *An. culicifacies,* 100% reduction in immature density was obtained with a dosage of 200 and 300 g a.i./ha respectively. At lower doses—@ 100 g a.i./ha, 76 to 87% reduction was observed on Day 7 in small shallow water bodies. At higher doses—@ 400 and 500 g a.i./ha, 100% reduction was recorded up to three weeks. In paddy fields and irrigation channels, with anopheline breeding consisting mainly of *An. culicifacies* and *An. subpictus*, 100% reduction of immature density was observed @ 300 g a.i./ha. In wells used for irrigation purpose, supporting breeding of *An. culicifacies* and other species, 93 to 96% reduction of anopheline immatures density was observed @ 300 to 500 g a.i./ha, while cent per cent reduction was observed @ 1000 g a.i./ha. In cement tanks where *An. stephensi* and *An. subpictus* breeding was observed, 100% reduction in density/dip was observed @ 100 to 200 g a.i./ha.

An. stephensi breeding was also commonly observed in waste commodes (WCs) in Goa. The efficacy of pirimiphos-methyl against breeding of *An. stephensi* in WCs, was also determined @ 100 and 200 g a.i./ha. Results revealed > 80% reduction @ 100 g a.i./ha as against 100% reduction @ 200 g a.i./ha up to one week (Table 2).

These trials clearly indicate that in fresh water habitats of *Anopheles* spp pirimiphos-methyl @ 200 g a.i./ha will be required to obtain 80 to 100% reduction invariably in all the habitats at weekly intervals.

Results of field trials against *Culex* species, carried out in polluted pools and muddy pits, unused wells and drains are given in Fig. 2 and Table 3. The efficacy of pirimiphos-methyl against immatures of *Culex* species was determined at different doses ranging from 50 to 1000 g a.i./ha. Results revealed almost 100% reduction in the density of immatures at a dos-

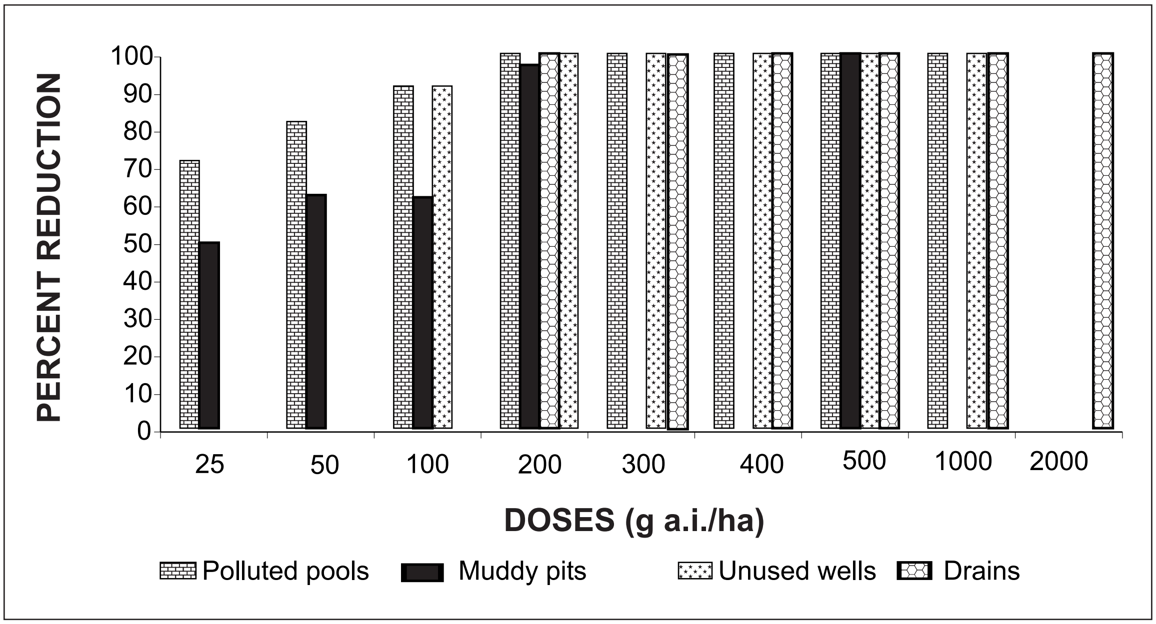

Fig. 2: Evaluation of pirimiphos-methyl 50% EC against immatures of *Culex* spp under field conditions in District Ghaziabad

Table 3. Bioefficacy of pirimiphos-methyl 50% EC against culicine immatures at different doses

Habitats	% Reduction on Day 7						
	50 g a.i./ha	100 g a.i./ha	200 g a.i./ha	300 g a.i./ha	400 g a.i./ha	500 g a.i./ha	1000 g a.i./ha
Polluted pools	27.6	47.3	74.6	93.1	100	–	–
Muddy pits	39.2	78.4	86	95.4	100	–	–
Unused wells	–	59.7	74	96.9	97.9	98.4	100
Drains	44.1	61.4	90.4	95.8	100	–	–

Note : Surface area : Pools (8 to 21.1 m^2); Muddy pits (3.4 to 4 m^2); Unused wells (1.69 to 3.3 m^2); and Polluted drains (79 to 110 m^2).

age of 200 g a.i./h with in 24 h after the treatment in different habitats (Fig. 2). However, the reduction was even < 80% after one week but > 80% reduction was obtained at the dose of 300 g a.i./ha and above in all the habitats (Table 3). In unused wells supporting *Culex* breeding, 100% reduction in the density of immatures was observed at a dose of 300 g a.i./ha. At higher doses similar impact, however, persisted for three days to four weeks. In polluted drains supporting *Culex* breeding, 90% reduction was observed on Day 4 @ 200 g a.i./ha and higher doses. These trials clearly indicate that in case of culicine species in different types of polluted water habitats, 90–100% reduction in the density of immatures can be obtained @ 200–400 g a.i./ha by weekly application of the larvicide. These results also suggest that pirimiphos-methyl is more effective against anopheline immatures in clear water than culicine immatures in polluted water habitats.

The efficacy of any larvicide varies against different species. At present temephos and fenthion are used as larvicides against anopheline and culicine breeding in fresh and polluted water habitats respectively. Though, temephos has a very low toxicity against mammals and has also been found to be highly effective as larvicide against *Culex* species in polluted water, it is not preferably used against culicines, because of its wider spectrum of cross-resistance to different organophosphorus insecticides caused by elevated esterases. Similarly, another larvicide chloropyriphos (Dursban) was also found to be highly effective against *Culex* larvae[13], but it has a very high toxicity against mammals and it also has a wider spectrum of cross-resistance to different organophosphorus insecticides. On the contrary, fenthion which does not show cross-resistance to other organophosphorus compounds with broad spectrum of cross-resistance is preferably used as a larvicide against culicines. A recent study carried out in Delhi, however, showed the possible development of resistance to fenthion in *Cx. quinquefasciatus* larvae[1]. The results of the present study also throw light on these observations.

Laboratory bioassays carried out to determine toxicity of pirimiphos-methyl against *G. affinis* revealed that pirimiphos-methyl is not toxic to fish at a concentration of < 0.25 ppm, as no mortality was observed at any of these concentrations tested up to72 h. However, at higher concentrations there was some mortality in larvivorous fish in the laboratory bioassays and therefore, doses higher than 0.25 ppm are not safe to this non-target species (Table 4). Toxicity test of pirimiphos-methyl to the main non-target organism—*G. affinis* which is being used extensively under EMCP, suggest that this larvicide should not be used at concentration above 0.25 ppm in habitats harbouring the larvivorous fish.

Table 4. Toxicity of pirimiphos-methyl 50% EC against the larvivorous fish, *G affinis* under laboratory conditions

Dose (ppm)	No. of fish exposed	% Mortality after		
		24 h	48 h	72 h
1.0	100 (4)	24	24	24
0.5	100 (4)	12	12	12
0.25	100 (4)	4	4	4
0.125	100 (4)	0	0	0
0.0625	100 (4)	0	0	0
0.3125	100 (4)	0	0	0
Control	100 (4)	0	0	0

Figures in parentheses indicate the number of replicates (25 fish in each replicate).

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