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FIELD EVALUATION OF PIRIMIPHOS-METHYL AS A MOSQUITO LARVICIDE IN AN URBAN AREA OF INDIA AS PART OF THE NATIONAL MALARIA ERADICATION PROGRAMME

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ABSTRACT. Following the resurgence of malaria in India, which was partially the result of an increased incidence of urban cases, alternative control methods against the vectors in urban areas were examined and adopted. During the continued examination of new insecticides, pirimiphos-methyl was tested and shown to be effective as a larvicide when applied each week at a rate of 12.5g active ingredient/hectare. Anti-larval operations with this treatment carried out in the urban areas of one large town resulted in a reduction of the transmission of malaria, lowering of the incidence among children, and decreasing the *Plasmodium falciparum* infection.

INTRODUCTION

As a result of intensive indoor spray treatments by the National Malaria Eradication Programme (NMEP) the incidence of malaria in India had been reduced by 1965 to less than 100,000 cases per annum. There was, however, a resurgence of malaria in the following years such that in 1975 over 5 million cases were recorded. The increased incidence of urban cases, and the development of strains of the vectoring species resistant to those insecticides in use, contributed to this resurgence. For example, after the use of BHC and DDT for many years large areas of India contain strains resistant to these insecticides, and even double resistance to both BHC and DDT occurs in *Anopheles culicifacies* (Brown & Pal 1971). In some areas where malathion has been used as a replacement, there is increased tolerance to this insecticide. (Rajagopal 1977.)

Malaria control methods in rural and urban areas are different; those in the former have predominantly used indoor spraying with residual insecticides to interrupt the transmission of malaria. In urban areas emphasis is placed on mosquito control using intensive anti-larval operations. For the successful control of malaria in areas where vector strains have acquired resistance to those insecticides used as indoor sprays, emphasis is now placed on finding alternative methods of control, one such being the use of appropriate larvicides in the rural parts of arid and semi-desert areas during pre- and post-monsoon periods.

In 1973 the urban malaria control scheme of NMEP was initiated, and during 1978 intensified anti-larval operations were used in 102 towns which had a high incidence of malaria. Both established and new larvicides are used in this urban malaria control scheme, the larvicide used depending on the type of larval habitat. In India the established larvicides are mosquito larvicidal oil, an emulsion concentrate of pyrethrum extract, Paris green, fenthion and temephos.

One new larvicide, pirimiphos-methyl, with a low mammalian toxicity comparable to temephos was examined in laboratory and field tests by Wattal et al. (1975). They found that satisfactory control of mosquito larvae was achieved with a dose of 12.5 gms active ingredient (ai) per ha., temephos giving comparable control at 25 gms ai/ha. The present study was undertaken in 1976 and 1977 to examine the impact of the use of this larvicide on the incidence of malaria in the town of Bhiwani in Haryana State.

EVALUATION AREA

Bhiwani is approximately 135 km from Delhi and has a population of almost 92,000 as established by the NMEP Census of 1975. It lies in the semi-desert plain area, the annual rainfall from 1974 to 1977 ranging from 296 mm to 667 mm. Monthly rainfall and the number of rainy days per month are given in Table 1. Recently a canal has been constructed and, in addition to water from this canal being used for irrigation, water is impounded in huge tanks from which it is piped to the town. Earlier, in the absence of a piped water supply water was drawn from a large number of wells within the town. The water supply is intermittent, and therefore water for domestic use has to be stored in containers where mosquito breeding can occur. The many disused wells also provide breeding sources. The drainage system is faulty, and frequently the drains have a poor gradient, so that stagnant pools are formed providing favorable conditions for intensive culicine mosquito breeding. Both *Anopheles culicifacies* and *An. stephensi* are known to transmit malaria in the evaluation area, the first species breeding in fresh rain water pools or canal water, the second in pitchers, water storage tanks and wells.

There are 3 textile mills on the periphery of the town, and these draw labor from adjoining districts and states outside the evaluation area. Laborers from these mills could contract malaria outside the treated areas, but those with malaria would report to the hospital in Bhiwani. As the untreated control area, the town of

Table 1. Rainfall of Bhiwani from 1974 to 1977.

	1974		1975		1976		1977	
	No. of rainy days	Rainfall (mm)	No. of rainy days	Rainfall (mm)	No. of rainy days	Rainfall (mm)	No. of rainy days	Rainfall (mm)
January	—	—	3	22	3	8	3	11
February	—	—	1	1	2	14	—	—
March	2	12	3	11	2	2	1	2
April	1	12	—	—	1	3	4	34
May	1	17	3	5	4	31	1	18
June	4	55	3	36	6	81	1	17
July	13	108	14	169	9	183	16	241
August	5	33	8	98	16	313	10	267
September	3	41	10	81	2	32	1	4
October	2	6	3	19	—	—	—	—
November	—	—	—	—	—	—	—	—
December	3	12	—	—	—	—	—	—

Devsar, with a population of approximately 5,000 and situated 8 km from Bhiwani, was used.

MATERIALS AND METHODS

A complete survey of mosquito breeding sites was carried out before the start of any larvicide application. Existing and potential breeding sites were identified and a plan of operation mapped out. The town was divided into 2 sectors, and each sector into 6 sections. Application of pirimiphos-methyl was made with knapsack sprayers, the application rate being 12.5 g ai/ha at weekly intervals. No attempt was made to estimate the volume of water in any breeding site, so that the concentration of active ingredient in the water varied. Domestic water, such as pitchers, tanks and overhead cisterns where breeding occurred, was not treated. Attempts to control breeding in such water collections by source reduction, i.e. persuading the householder to reduce or remove such sites, were not successful. No residual insecticide focal sprays were applied; neither was a barrier spray with a residual insecticide used on the periphery of the evaluation area.

The entomological evaluation included assessments of both adult and immature populations. Eighty adult catching stations were established, and on each week day 16 catching stations were visited and the indoor adult mosquito population collected with an aspirator. In addition, 100 mosquito larval sites were examined each week, 20 sources being checked each day. For these larval assessments, 5 dips were made with a standard size ladle, and the average number of 3rd and 4th instar larvae, and also pupae, were recorded. General mosquito control by the use of the larvicide was one of the objectives of the programme and thus those mosquitoes collected, were only identified as either anopheline or culicine.

Devsar, the control village, was divided into 5 sections, and adult and larval collections made similarly to those at Bhiwani. House to house malaria surveillance in both Bhiwani and Devsar, already in force as part of the NMEP was continued and used for epidemiological evaluation of the treatment. In both towns all fever cases received presumptive treatment with chloroquine. Positive malaria cases, including cases of falciparum malaria, were given 5 days radical treatment with primaquine.

RESULTS

Treatments commenced on 18 August 1976, but the programme did not become fully operational until September and ended in November 1977. The collection of entomological data in the untreated control area started in October, since the initial control site had to be abandoned. Average larval and pupal incidences were calculated, and Table 2 shows these as monthly totals. Similarly, average adult mosquito collections were determined, and again these are presented on a monthly basis in Table 2. The epidemiological data from both treated and untreated areas are presented in Table 3. This shows the monthly total of blood smears and fever cases examined, and also the numbers positive and the positivity rate. Positivity rates amongst infants (0 to 1 year of age) and children 1 to 5 years old are given in Table 4, and, to determine the impact of the larvicide on the incidence of infection with *P. falciparum*, Table 5 lists such cases on a monthly basis.

DISCUSSION

In arid and semi-desert areas not only is the amount of rainfall important, but also the number of rainy days, as the continued favorable temperature and humidity produce conditions conducive to the longevity of mosquitoes. Heavy rains fell in August 1976, 313 mm falling in 16 days, giving rise to innumerable mosquito breeding sites. The long period of rain provided favorable conditions for mosquito survival. As the project was not fully operational until September any de-

Table 2. Immature and adult mosquitoes collected in treated & untreated areas.

	Treated				Untreated			
	Av. No. immature		Av. No. Adult		Av. No. Immature		Av. No. Adult	
	Anopheles	*Culex*	*Anopheles*	*Culex*	*Anopheles*	*Culex*	*Anopheles*	*Culex*
August 1976	765	117.3	416	78	—	—	—	—
September "	85	10.7	550.8	97.4	880	10	111	8
October "	8	27	151.2	67.6	614	6	111	2
November "	7	10	47	58	351	0	154	2
December "	6	2	3	19	112	5	34	4
January 1977	0	0.5	0	4.5	1	32	0	0
February "	0	1.7	0	2.7	0	17	0	0
March "	0	8.5	0.2	8.5	0	253	0	3
April "	0	39.9	2.7	73.5	12	802	0	27
May "	1.6	42.8	6.9	73.7	12	239	6	13
June "	3.5	7.3	9.6	32	236	93	35	0
July "	72.6	8.8	278.7	40.8	1522	267	697	19
August "	174.2	33.7	640	106.5	3353	41	1739	2
September "	64.7	31.2	162	43	1685	0	372	6
October "	5.7	5.1	51	35	1026	0	324	1

Table 3. Number of blood smears examined, number positive and positivity rate for treated and untreated areas during 1976 & 1977.

	Treated			Untreated		
	No. Blood Smears Examined	No. Positive	Positivity Rate	No. Blood Smears Examined	No. Positive	Positivity Rate
January 1976	261	5	1.9	2	0	0
February "	705	36	5.1	112	1	0.9
March "	1072	67	6.3	119	2	1.7
April "	1172	184	15.7	93	6	6.5
May "	1273	412	32.4	131	6	4.6
June "	1623	588	36.2	120	20	16.7
July "	1598	674	42.2	70	46	65.7
August "	2070	726	35.1	155	45	29.0
September "	4962	1566	31.6	185	52	28.1
October "	3659	693	18.9	213	51	23.9
November "	974	144	14.8	168	26	15.5
December "	500	58	11.6	106	5	4.7
January 1977	356	49	13.8	148	10	6.8
February "	897	99	11.0	126	16	12.7
March "	1077	103	9.6	219	32	14.6
April "	2270	348	15.3	325	160	49.2
May "	3142	626	19.9	317	107	33.8
June "	3229	624	19.3	418	160	38.3
July "	3403	754	22.2	436	193	44.3
August "	4365	746	17.1	496	164	33.1
September "	4138	711	17.2	664	178	26.8
October "	2444	241	9.9	121	27	22.3
November "	1103	93	8.4	150	13	8.7

Table 4. Malaria positivity rate for infants and 1–5 year old children in Bhiwani for 1976 and 1977.

	% positivity			
	1976		1977	
	Infants	1–5 years old	Infants	1–5 years old
January	0	0	0	0
February	0	0	0	10.1
March	0	3.5	0	4.2
April	22.2	13.5	0	7.2
May	0	17.1	2.7	6.2
June	13.6	15.7	2.1	8.8
July	17.7	28.1	0	6.9
August	20.0	27.5	0	7.2
September	9.2	16.7	6.1	8.6
October	15.1	14.7	0	1.8
November	0	8.5	0	5.3
Total blood smears examined	271	1455	306	4498
% positivity	12.6	16.0	1.6	8.3

crease in mosquito breeding sites only occurred from that date, and since measures were not taken against adults either within the town or as a barrier spray, the mosquito population remained high and the anti-larval operations only produced a decline from October onwards.

During 1977 the anopheline density remained under control until July, but with the onset of rains it showed a rise, reaching a peak in August. July and August 1977 had 241 mm and 267 mm rain, and 16 and 10 rainy days, respectively. Due to the innumerable breeding sites that developed outside the periphery of the treated area, infiltration of anophelines occurred giving rise to an increase in the anopheline population within Bhiwani. Once the rains ceased at the end of August there was a decline in the anopheline density, the arid and semi-desert conditions helping to eliminate small collections of water and permitting the easier management of the larger pools. The culicine population, mainly breeding in areas with poor drainage showed a similar trend, although in general the numbers collected were lower than the numbers of anophelines in the samples for comparable months. The peak in adult culicine density also occurred in August in the treated area, whereas the peak larval culicine density in the untreated area occurred in April, adult culicine numbers being low in the untreated area, and there was no well defined peak. Culicine infiltration was negligible.

Table 5. Monthly incidence of *P. falciparum* cases in 1976 and 1977 in Bhiwani.

	1976		1977	
	Number	%	Number	%
January	3	1.15	2	0.56
February	0	0	1	0.11
March	0	0	1	0.09
April	0	0	0	0
May	2	0.16	0	0
June	0	0	0	0
July	1	0.06	0	0
August	1	0.05	5	0.12
September	10	0.20	0	0
October	20	0.55	2	0.08
November	3	0.31	1	0.09
December	2	0.40		
Total	42	0.21	12	0.05

The high positivity rate from May to September 1976 (Table 3) indicated a large reservoir of parasite infection in the

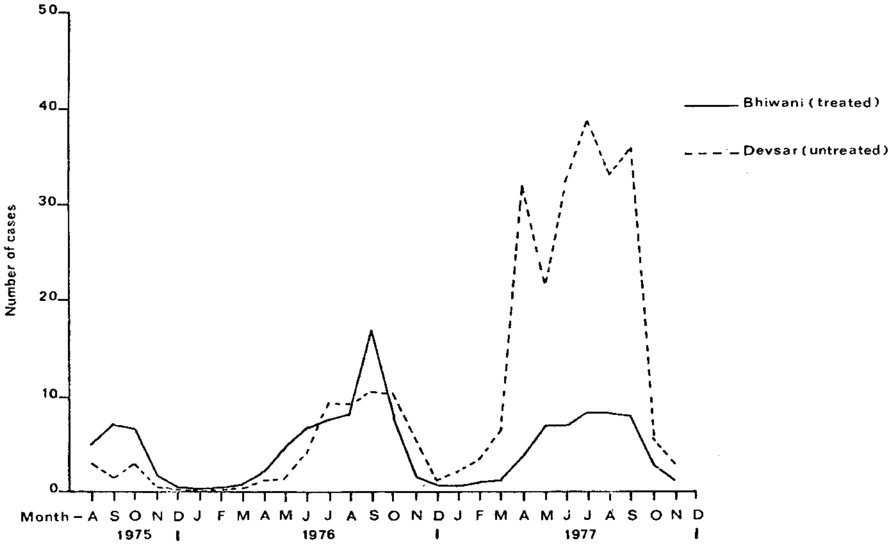

Fig. 1. Average number of malaria cases per 1000 population of Bhiwani and Devsar.

community at the beginning of the project. In the absence of adult control measures, no immediate effect on the interruption of malaria transmission was anticipated, and in fact any transmission occurring at the beginning of the project could have continued. The measures were expected to keep the anopheline population density under control so that transmission would not be possible or would only continue at a low level. That transmission at a low level was possible is borne out by the following figures for positivity rates for 1976 and 1977 in Bhiwani.

	1976	1977
June	36.2	19.3
July	42.2	22.2
August	35.1	17.1
September	31.6	17.2
October	18.9	9.9

However, in the control village of Devsar the incidence of malaria remained high from April 1977 onwards due to high densities of vector populations produced from the numerous breeding sites. The decrease in positivity rate in the control village during November 1976 to February 1977 and in October and November 1978 was due to the general absence of transmission during this time. This can be seen in Table 3 and Figure 1.

Analysis of the malaria incidence in infants and children up to 5 years old given in Table 4 shows that very few infants were found positive during 1977, and a low level of positivity occurred only in 3 months of that year. The annual positivity rate was only 1.6%. Further, there was a 50% reduction in the incidence in children, the positivity rate decreasing from 16.0 in 1976 to 8.3 in 1977 despite the number of blood smears examined in 1977 being three times higher than in 1976. A significant decrease was seen in the incidence of *P. falciparum* infection, from 0.21% in 1976 to 0.05% in 1977 (Table 5).

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A COMPARISON OF EGG HATCHING TECHNIQUES FOR THE WESTERN TREEHOLE MOSQUITO, *AEDES SIERRENSIS*

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ABSTRACT. Aqueous solutions of 0.01% ascorbic acid, 0.15% cysteine hydrochloride, 0.2% Bacto-Peptone, 1.0% Na_2SO_3, and water deoxygenated by boiling and bubbling N_2 were used as stimuli to hatch eggs of 5 California strains of *Aedes sierrensis*. The 1.0% Na_2SO_3 solution produced the best mean hatch (84%) for all strains but high mortality of larvae occurred when they were left in this solution for 24 hr. By exposing eggs to 0.1% Na_2SO_3 for 3 hr, then diluting with fresh water, negligible larval mortality occurred and there was no change in the percent egg hatch. Considerable differences in hatching success were observed between some strains exposed to the same hatching stimuli, and between given strains exposed to different hatching stimuli.

The western treehole mosquito, *Aedes sierrensis* Ludlow, is one of the main suspected vectors of the dog heartworm in California (Weinmann and Garcia 1974, 1975), as well as a common pest of humans and other vertebrates. It is difficult to reduce populations of this mosquito because it develops in cryptic tree-hole habitats that are difficult to find and eliminate as breeding sources. For this reason the sterile male technique (Bushland 1971) is being investigated as one possible alternate method of control. However, before we can realistically consider the release of sterile males as one component of an integrated pest management program for area wide reductions in populations of this mosquito, we need to develop an effective and economic standardized method for mass producing it. One requirement for a mass rearing program is that the eggs of *Ae. sierrensis* can be consistently hatched with good success in the laboratory. Hatching of aedine mosquito eggs is stimulated by a reduced dissolved oxygen concentration (DOC) in the hatching medium (Gjullin et al. 1941), and numerous methods to achieve a low DOC and stimulate egg hatching are known (e.g. Gjullin et al. 1941, Borg and Horsfall 1953, Barbosa and Peters 1969). The purpose of this study was to determine which method produced the best egg hatch and to determine if different strains of *Ae. sierrensis* responded differently to the same hatching stimulus.

MATERIALS AND METHODS

The 5 strains of *Ae. sierrensis* studied originated from the following sources: a