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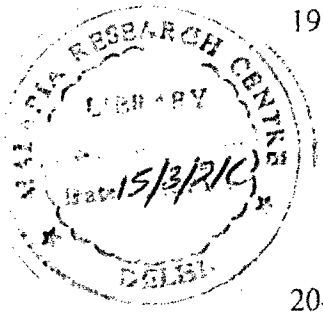

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Extended Field Trial of Deltamethrin WDP for Control of Malaria at Jagdalpur, Madhya Pradesh, India

K.S.GILL, S.J. RAHMAN, R. PANDA[a], KAUSHAL KUMAR and R. KATYAL

The synthetic pyrethroid, deltamethrin 2.5% wdp, was evaluated at a dosage of 25 mg/sq m in a group of villages of tribal area around Jagdalpur, District Bastar (Madhya Pradesh). Study was carried out in three consecutive years from 1986 to 1988 with a total of five rounds of deltamethrin spray covering a population of approximately 10,000. The suspension of deltamethrin wdp was white in colour, odourless, non-irritant and the deposits were faintly visible on the surface of wall. A total of 3848 kg of 2.5% deltamethrin wdp was consumed during the trial. Insecticide was found effective in keeping the adult vector density *An. culicifacies* to a very low level for a period of 14-16 wks. Both anopheline and culicine mosquitoes were affected by the spray. The trial confirmed its long residual effectiveness from 15 to 16 wks on both mud and cement plastered surfaces. Deltamethrin spray shortened the average life span of vector mosquitoes. Observations indicated that insecticide although has an excito-repellent effect on mosquitoes, thus forcing exodus from treated area but with poor survival. Partial impact of deltamethrin spray was observed on the larval population confirm this. Deltamethrin spray did not exhibit any fumigant effect on mosquitoes.

Significant decline in SPR from 17.93 to 4.26% was observed in areas sprayed with deltamethrin as compared to the comparison area. Similarly, SfR showed 81% reduction in experimental area while the control area showed increase by 42 per cent. No ill-effect of spray was noticed on the inhabitants, spray personnels, animal population and other non-target organisms of the area. The acceptability of the deltamethrin spray was found to be very high.

Keywords: Deltamethrin, Insecticide, Jagdalpur, Malaria control

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INTRODUCTION

Development of resistance of the malaria vector species to conventional insecticides stimulated the need for evaluating newer group of insecticides[1,2]. In recent years, Globally, several photostable pyrethroids such as permethrin, fenvalerate, alphamethrin, fenfluthrin, cyfluthrin, cypermethrin, deltamethrin, etc., have been evaluated as a larvicides and adulticides[3-9]. In India, there are few laboratory and field trials which evaluated the entomological impact of deltamethrin against various mosquito vectors[10-12].

In order to see the impact on malaria transmission by reducing the vector density through residual spray of deltamethrin, a large scale field trial was carried out in a group of villages around Jagdalpur, District Bastar (M.P.) from July 1986 to March 1989. Results of four years of study are presented in this paper.

Study areas

The trial was carried out in a highly endemic area for malaria in a tribal belt of Jagdalpur, District Bastar (M.P.). The area showed persistent malaria transmission in spite of many decades of spraying of DDT/HCH under NMCP/NMEP. *Plasmodium falciparum* was the predominant malaria parasite in this area[13].

Area selected for trial under Jagdalpur NMEP unit were, (i) Kumrawand, (ii) Tokapal, and (iii) Kurandi (Fig. 1). Ten villages of Kumrawand and Tokapal area with a total

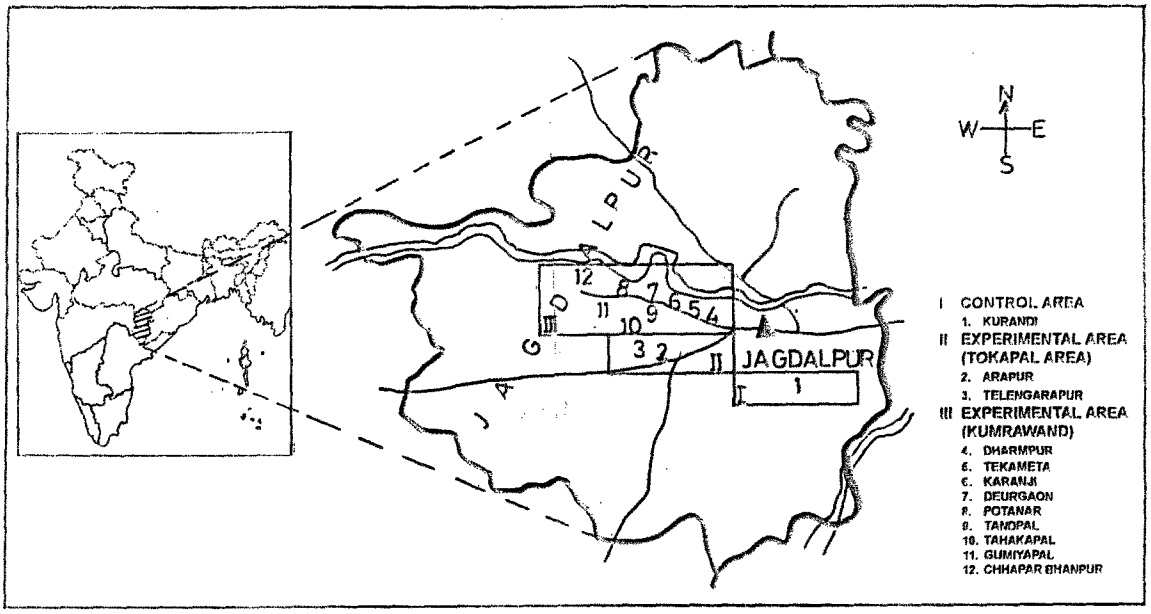

Fig. 1. Map of the area for group of villages scale field trial at Jagdalpur, M.P.

population ranging from 9000 to 10,000 were selected for deltamethrin spray and were named as experimental villages/area. The control area was comprised of a village and its hamlets in Kurandi area with a population of 2000 to 3000.

The climate of the area remains moderately hot and moist throughout the year. During the trial period from 1986 to 1988, the mean minimum and maximum temperature ranged from 14.24 to 34.96°C, whereas, the relative humidity ranged from 48.33 to 91.40 per cent respectively.

Mosquito fauna of the area comprised of 19 anopheline species. Among these anophelines, the major vector species present were *An. culicifacies, An. fluviatilis, An. stephensi* and *An. annularis*. Culicine fauna of the area mainly comprised of *Culex vishnui* group of mosquitoes. Malaria transmission in the area generally takes place from July to October with maximum incidence of malaria in the month of August and September.

All the selected experimental and control villages were covered under NMEP with two rounds of DDT spray till 1970. From 1970 onwards, DDT was substituted with three rounds of HCH annually.

MATERIALS AND METHODS

Spray operation

Deltamethrin (2.5% WP) was sprayed at the dosage of 25 mg/sq m. The insecticide was supplied through the courtesy of M/s. Roussel Pharmaceuticals India Ltd. The spray was carried out from 1986 to 1988 with a total of five rounds of spray in three consecutive years. The control area was sprayed as usual with three rounds of HCH annually at the dosage of 20 mg/sq ft. Spray coverage exceeded 85% in both the areas. Various protective clothing, viz. thick overalls, rubber gloves, goggles, masks etc., were provided to the spraymen during the spray operation.

Entomological evaluation

Two villages (referred as index villages) were selected for study in each of the experimental and control areas. Various entomological parameters monitored in Index villages before and other the spray at weekly/fortnightly intervals included, (i) indoor catches; (ii) outdoor catches; (iii) night collection by CDC light-trap; (iv) parity rates; (v) impact on larval population; (vi) bioassay, contact and airborne; and (vii) susceptibility test as per WHO standard techniques.

Toxicological evaluation

Toxic effect of the insecticide on inhabitants/spray personnel and acceptability of the spraying was observed by asking various quaries from the local populations of the area. Other collateral benefits of the insecticide and effect on other non-target fauna were also noted.

Parasitological evaluation

Parasitological evaluations was carried out

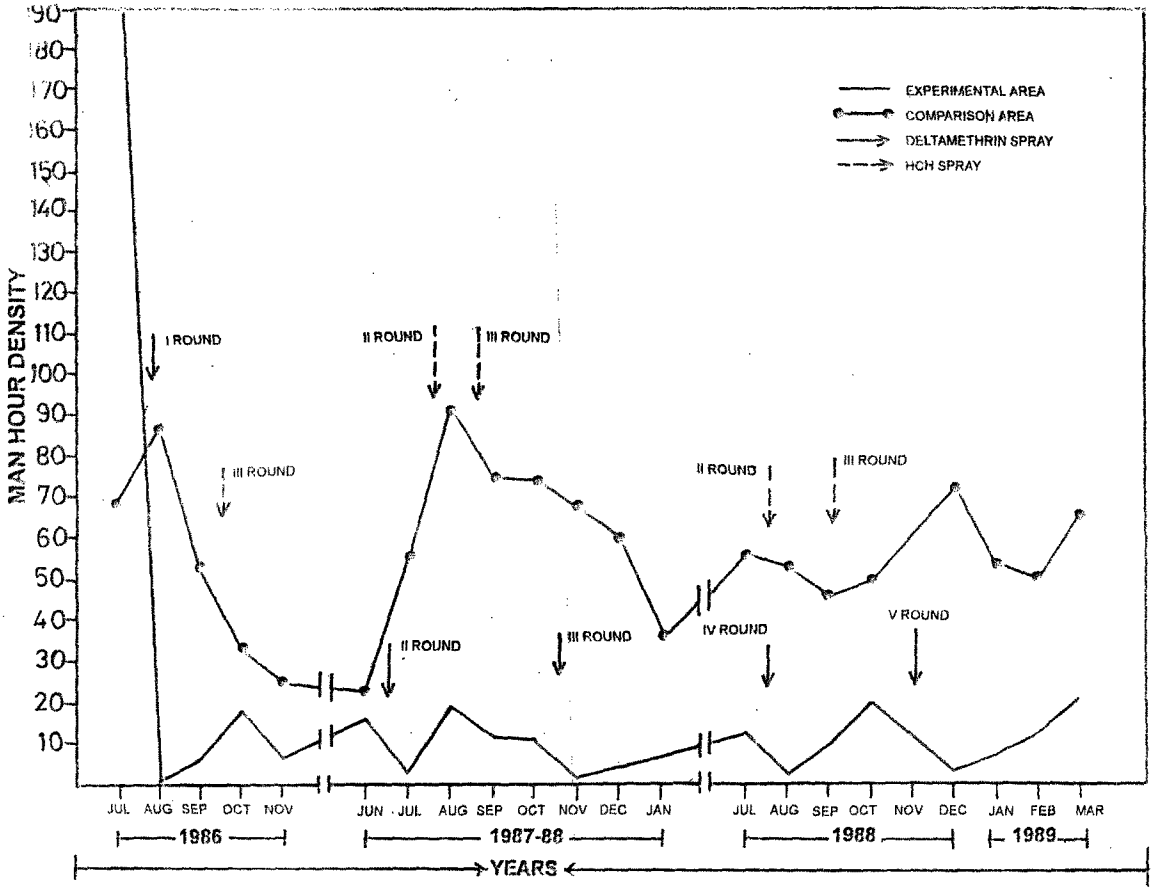

Fig. 2: Indoor man hour density of anopheline mosquitoes

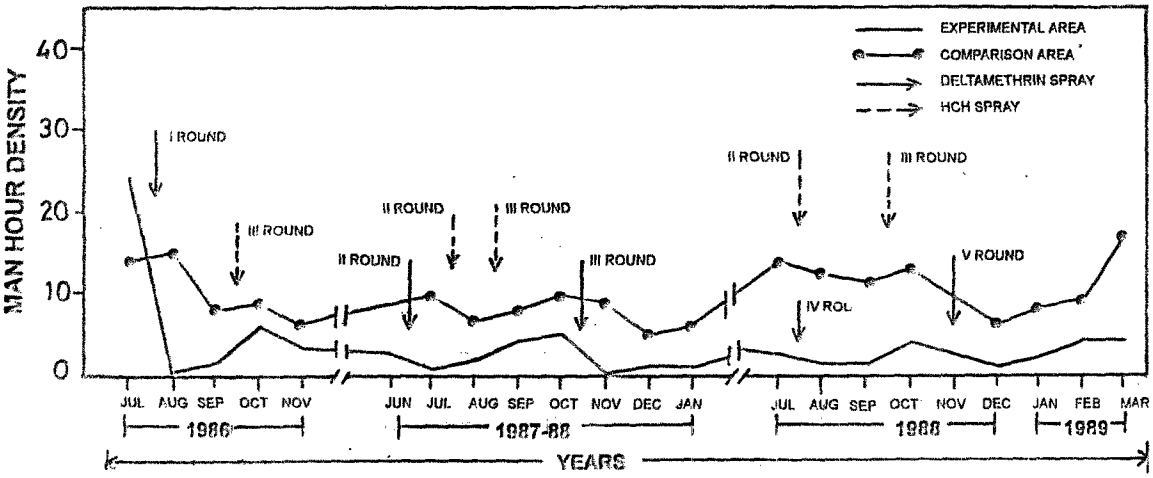

Fig. 3: Indoor man hour density of culicine mosquitoes

fortnightly by monitoring malaria by active case detection in the index villages. Blood smears were collected regularly from all the fever cases both from experimental and comparison areas. Presumptive treatment followed by radical treatment in positive cases was given as per NMEP schedule. Year-wise slide positivity rate (SPR), slide falciparum rate (SfR), blood examination rate (BER) and parasite incidence (PI) were calculated before and after the spray.

RESULTS AND DISCUSSION

Entomological evaluation

The data on various entomological parameters used for evaluating the effectiveness of insecticide both from experimental and comparison areas are presented and discussed as follows:

Mosquito density

Indoor mosquito density: Man hour density (MHD) of all anophelines, all culicines and *An. culicifacies* mosquitoes are presented graphically in Figs. 2-4. The pre-spray MHD of anopheline and culicine mosquitoes in experimental area before the deltamethrin spray in July 1986 were 187 and 24, whereas in comparison area, the corresponding values were 68 and 14. After the spray of first round of deltamethrin in experimental area, the MHD of anopheline and culicine mosquitoes declined drastically up to four months and remained in between 0 to 20 and 0 to 10, respectively. Thus, a reduction of 86 to 100 per cent in anopheline and 58 to 100 per cent in culicine mosquitoes density was observed up to 16 wk. On the contrary, in the comparison area which was being sprayed with three rounds of HCH annually, there was an increase in the MHD of mosquitoes followed by natural decline (Figs. 2 and 3). Similar trend was obtained in the MHD of *An. culicifacies* the vector species in experimental and control areas after the spray (Fig. 4).

Same pattern was observed in the decline of MHD of anopheline and culicine mosquitoes after subsequent rounds of deltamethrin spray in the experimental area. The impact of HCH spraying on the density of mosquitoes in comparison area was very less that too for shorter duration. Build up of mosquitoes density before the next round of deltamethrin spray in experimental area was comparatively much lower than the density in the comparison area.

Thus, the above results clearly indicate that deltamethrin at a dosage of 25 mg/sq m was very much effective in reducing the mosquitoes density to a very low level up to 16 wks. However in another study in India, during the field evaluation of deltamethrin at the dosage of 20 and 25 mg/sq m in a group of villages field trial in the District Ghaziabad (U.P.), reported effective control of DDT/HCH resistant *An. culicifacies* up to a period of 8 wks only after each round of spray[12]. Outside India, in Nigeria, during the field evaluation of deltamethrin at a dosage of 50 mg/sq m, satisfactory reduction of *An. gambiae* and *An. funestus* densities for a

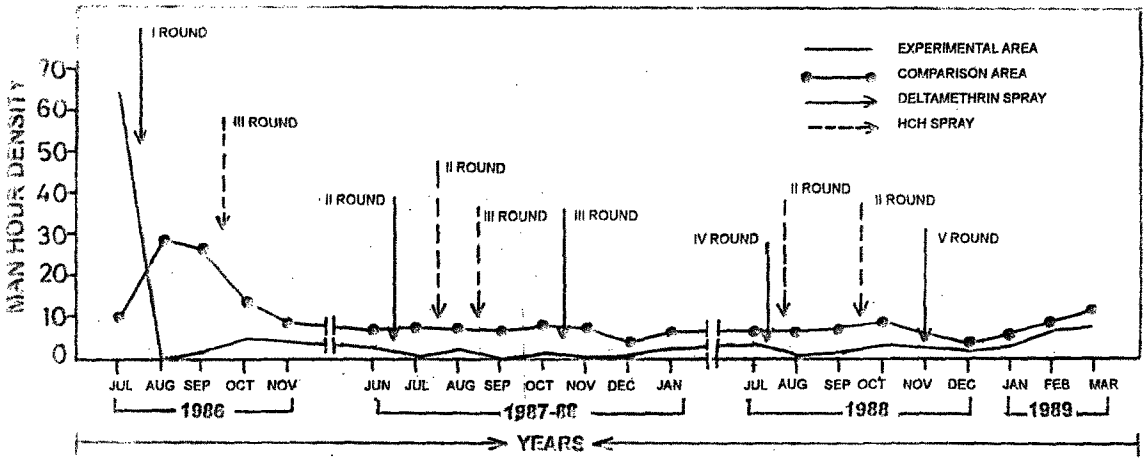

Fig. 4: Indoor man hour density of *An. culicifacies*

period of 12 to 20 wks was observed[9]. Similarly, in upper Volta, the effectiveness of decamethrin was observed for a period of one month against *An. gambiae* and *An. funestus* when tried at a dosage of 25 and 50 mg/sq m[6].

Outdoor mosquito density: Results of the impact of deltamethrin spray on the outdoor man hour density (MHD) of anopheline mosquitoes of experimental and comparison areas are presented graphically in Fig. 5. The pre-spray MHD of outdoor anopheline mosquitoes before the I round of spray in experimental area was 9. Immediately after the spray, outdoor MHD of anopheline started increasing from 9 to 15 for a period of 8 to 9 wks. The anopheline species collected and includes both vector and non-vector species. There was no change in the outdoor MHD of culicine mosquitoes in the experimental area, most probably due to low culicine density and their outdoor habits and habitats. In comparison area, the outdoor MHD of

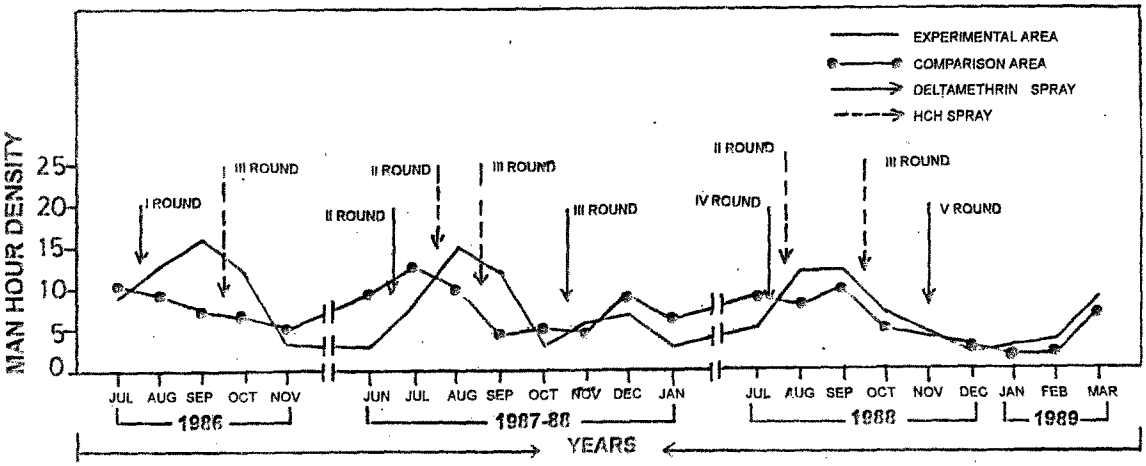

Fig. 5: Outdoor man hour density of anopheline mosquitoes

anopheline and culicine mosquitoes varied with the influence of natural climatic factors. Same was the impact of subsequent of deltamethrin spray.

From the results, it appears that the increase in the MHD of outdoor anopheline mosquitoes might probably be due to the irritant effect of deltamethrin only on the fraction of the mosquito population, forcing mosquitoes to migrate outside the sprayed area. Similar observations were made by the authors during the village scale field trial at Loni PHC (U.P.), India[10] and by Rishikesh *et al.*[9], during the evaluation of deltamethrin in Nigeria.

Parous rate: In order to compare the impact of deltamethrin and HCH spray on the longevity of the anopheline mosquitoes, dissection of *An. culicifacies* mosquitoes were carried for parity rate (Fig. 6). The parous rate of *An. culicifacies* in experimental area before deltamethrin spray was 73 per cent. Immediately after the first round of deltamethrin spray, the parous rate during the first month could not be assessed because sufficient number of vector mosquitoes were not available for dissection. During the second and third month after spray, the average parous rate of vector species in experimental area was found to be varied between 41 and 52 per cent, while the similar figures for the comparison area during the corresponding months was 60 to 71 per ent. Similar trend was obtained during the subsequent rounds of deltamethrin spray.

The above data reveals that the parous rate of *An. culicifacies* in experimental area dropped considerably as compared to HCH

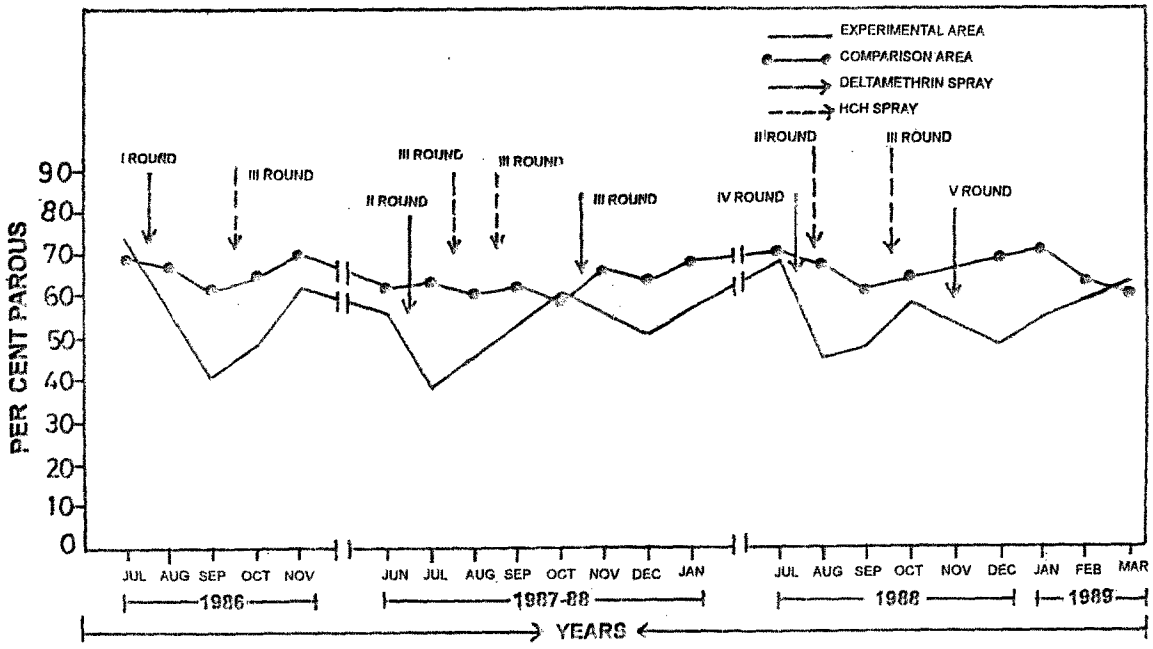

Fig. 6: Parous rate of *An. culicifacies*

sprayed area. Further, a significant difference was also noticed in the parous rate of *An. culicifacies* in the experimental area before and after the spray for a period of 8 to 12 wks. Thus, the results of this study clearly establish that the residual spray of deltamethrin shortened the average life span of the vector mosquito. Similar, observations were made during the evaluation of deltamethrin in Nigeria, where a significant decline in the parity rate of *An. funestus* was noticed in the experimental village[9].

Light-trap collection: The pre- and post-spray data on the number of anopheline and culicine mosquitoes collected by light-traps (average per trap per night) from both the areas are given in Figs. 7 and 8. The pre-spray collection of light-traps showed that more number of culicine mosquitoes were attracted towards light as compared to anopheline mosquitoes. The anopheline mosquito collected were mostly represented by other non-vector species. The culicine fauna was mainly represented by *Cx. vishnui* group of mosquitoes.

The number of anopheline and culicine mosquitoes collected in light-traps before spray in experimental area were 21 and 188 respectively per night per trap. After the I round of deltamethrin spray, the average number of anopheline mosquitoes collected during the first and second month after the spray were 40 and 110. The increase in the number of anopheline mosquitoes in the light-

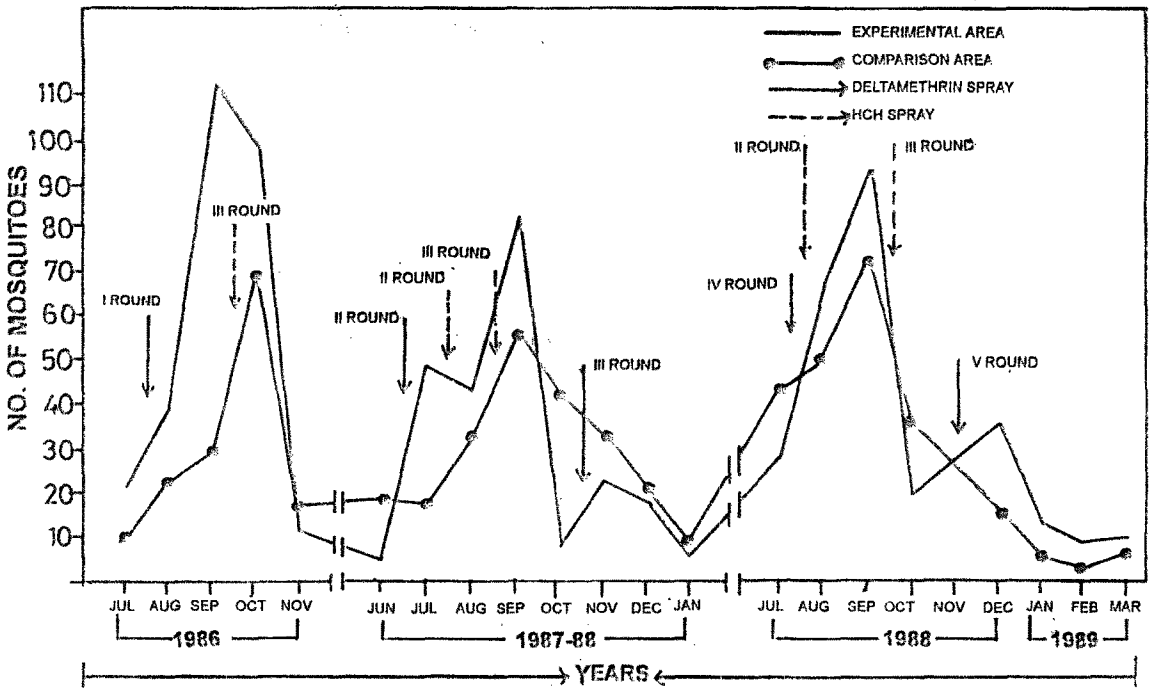

Fig. 7: Number of anopheline mosquitoes collected by light-traps (average per trap/night)

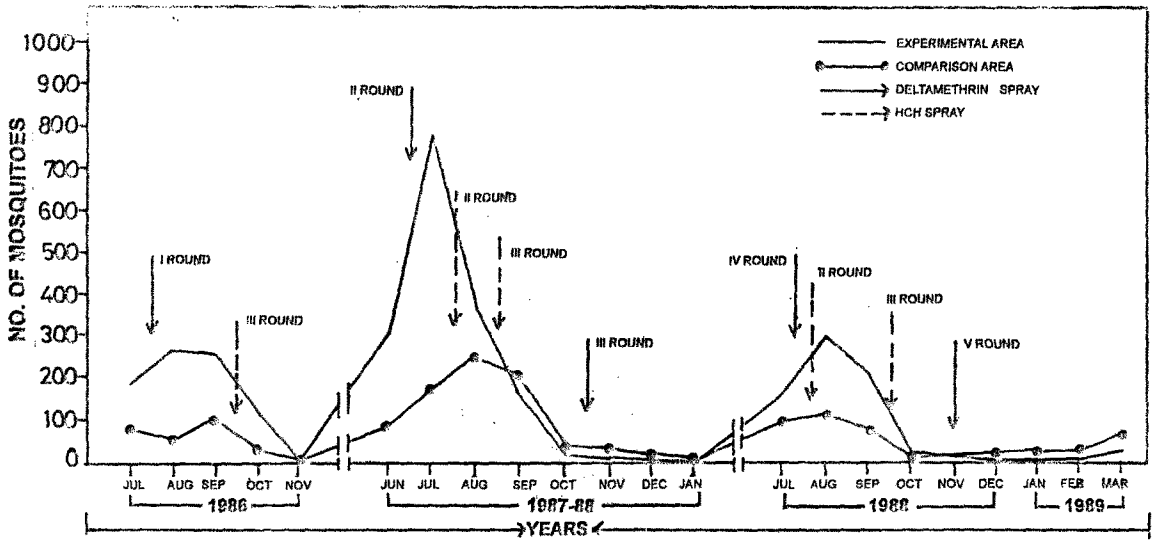

Fig. 8: Number of culicine mosquitoes collected by light-traps (average per trap/night)

traps was observed in the first two months after the deltamethrin spray. No extra-domiciliary resting of *An. culicifacies* was found during the day-time collection. Similar trend was observed during the subsequent rounds of spray.

This indicates although the vector species moved out due to excito-repellency but could not survive because of pick up of lethal dose. Unlike in DDT, after a few years of DDT spray, extra-domiciliary transmission was reported in *An. culicifacies*[14].

Effect on larval population: Impact of deltamethrin spraying on the larval population of mosquitoes is shown in Fig. 9. The pre-spray average number of anopheline and culicine larvae in experimental area were 6.6. After the first round of deltamethrin spray, the average number of anopheline and culicine larvae during the first month came down to 3.2. Thus, there was a reduction of about 50 per cent in larval population during the first month of deltamethrin spray. After this the impact on larval population started declining. On the other hand in comparison area, the impact of HCH spray on larval population was very little.

It appears from the above data that there is partial impact on mosquito larvae in the area sprayed with deltamethrin. In comparison area, no change was observed in the larval population of mosquitoes due to HCH spray.

Bioassay of the residual spray

Contact bioassay: Deltamethrin spray on mud and stone surfaces produced cent per

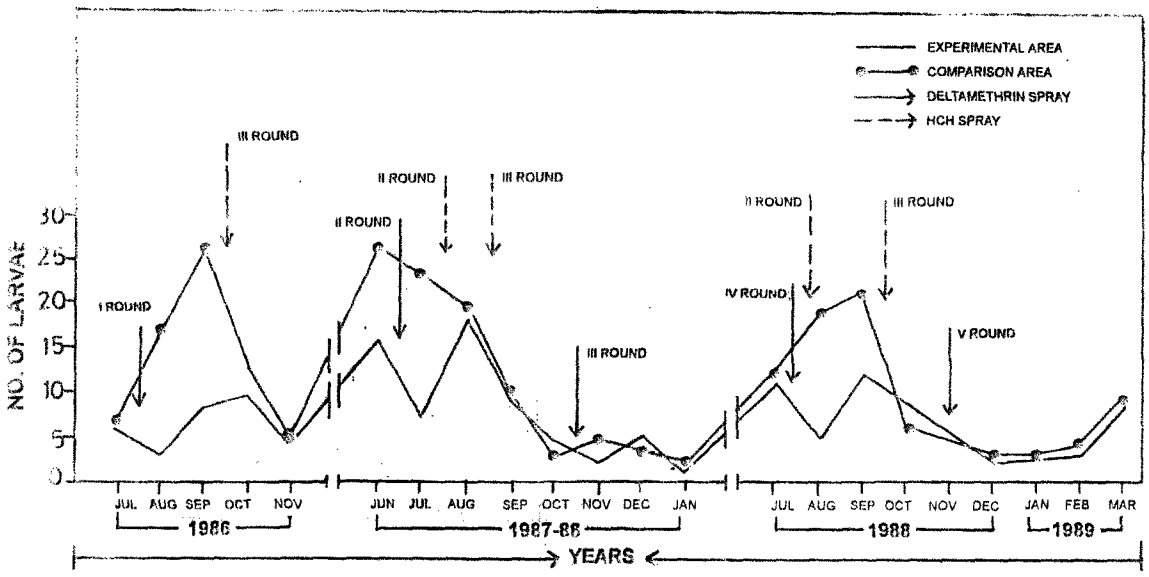

Fig. 9: Density of anophelines and culicines mosquito larvae (average per ten dips)

cent mortality in mosquitoes up to 7 to 13 wks after each round of deltamethrin spray. Subsequently, the mortality started declining. However, effective mortality of 58 to 86 per cent was observed for a period of 14 to 16 wks. The results of bioassay tests after the I round of deltamethrin spray is given in Table 1. On the contrary in the comparison area, the bioassay mortality, immediately after the HCH spray, was in the range 20 to 25 per cent that too only for 1 to 2 wks. During the study, interestingly, it was observed that even after mud plastering on sprayed surface, sufficient mortality was observed for a period of 4 to 6 wks. Almost same mortality was observed during subsequent rounds of deltamethrin spray.

The results of bioassay study clearly indicates that deltamethrin, sprayed at the dosage of 25 mg/sq m, had a residual toxicity of more than 14 to 16 wks on mud and stone surfaces. The residual effectiveness on stone surface was comparatively more than on the mud surface. Various authors reported different duration of residual effectiveness of deltamethrin when tried at different dosages. Residual effectiveness of deltamethrin for a period of 8 wks against *An. culicifacies* was observed at a dosages of 20 and 25 mg/sq m during a group of villages scale field trial[12]. But, the same insecticide when evaluated in a village scale field trial at a dosage of 12.5 mg/sq m gave a residual effect of 4 to 6 wks on mud and cement surfaces[11]. It is interesting to note that deltamethrin during the village scale trial at a dosage of 25 mg/sq m in Loni area of U.P. gave a residual potency up to 12 wks[10]. But, the same insecticide when tried at the same dosage in another part of India, Jagdalpur, District Bastar (M.P.), the residual effectiveness was found up to 14 to

Table 1. Results of contact bioasay tests performed in the experimental area after I round (18.7.86-30.7.86) of deltamethrin spray (1986)

Month/ week	Per cent mortality in mosquitoes		
	Mud surface	Stone surface	Control
August			
I week	100 (90)	100 (60)	0 (30)
II week	100 (60)	100 (60)	0 (30)
III week	100 (75)	100 (75)	0 (45)
IV week	100 (75)	100 (80)	1 (15)
September			
V week	100 (75)	100 (100)	3.3 (45)
VI week	–	–	–
VII week	100 (80)	100 (75)	2 (30)
VIII week	–	–	–
October			
IX week	95 (100)	100 (60)	0 (15)
X week	100 (75)	100 (85)	4 (50)
XI week	89 (45)	99 (85)	0 (15)
XII week	92 (60)	92 (60)	0 (30)
November			
XIII week	–	–	–
XIV week	86.6 (60)	89 (75)	0 (45)
XV week	79.7 (75)	74 (75)	3 (45)
XVI week	62.4 (60)	69 (45)	5 (50)

(–) data could not be collected; Figures in parentheses represent number of mosquitoes exposed.

16 wks. This appears to be correlated with environmental conditions of the area where considerable low temperature and high humidity prevails most of the time during the year. Moreover, most of the synthetic pyrethroids are found more active at low temperature.

In upper Volta, satisfactory residual effect of deltamethrin was found for 8 wks against *An. gambiae* and *An. funestus*, during the village scale evaluation at a dose of 25 mg/sq m[5]. But, the same authors reported higher residual effect of deltamethrin for a period 28 wks on mud surface with pronounced fluctuation at a higher dose of 50 and 100 mg/sq m[6].

A close scrutiny of the bioassay results obtained during various trials by various workers as well as results obtained during the present study indicates that irrespective of the dosage applied, the consistency of the bioassay results is more on the lower dosages. In view of the above, it appears that a dosage of 25 mg/sq m is the most appropriate dosage of deltamethrin when used as a residual insecticide on mud and other surfaces against Indian anopheline and culicine mosquitoes.

Air borne bioassay: No mortality was observed among mosquitoes which were kept in Barraud's cages inside the deltamethrin sprayed rooms for 24 h. Thus, it is evident that residual spray of deltamethrin do not have any fumigant effect.

Table 2. Epidemiological data of the deltamethrin trial at Jagdalpur, M.P. (1986-1988)

Year	Rounds of deltamethrin spray with period	Area/(Pop.)	No. of BS collected	No of BS (+)ve	SPR	SfR	BER	PI
1986	Pre-spray (Jan-Jun)	Experimental (4982)	579	104	17.96	16.75	11.62	20.87
		Comparison (4982)	307	31	10.09	9.44	10.03	10.13
	Post-spray after I round (Aug-Nov)	Experimental (4982)	1231	59	4.79	3.89	24.70	11.84
		Comparison (3060)	846	81	9.57	8.15	27.64	26.47
1987	Pre-spray (Jan-Jun)	Experimental (5056)	581	68	11.70	9.64	11.49	13.44
		Comparison (3422)	353	67	18.98	16.99	10.31	19.57
	Post-spray after II round (Jul-Sep)	Experimental (5056)	522	32	6.13	5.74	10.32	6.32
		Comparison (3422)	514	68	13.22	12.25	15.02	19.87
	After III round (Oct 87-Jan 88)	Experimental (5056)	477	21	4.40	3.98	9.43	4.15
		Comparison (3422)	409	33	8.06	7.09	11.95	9.64
1988	Pre-spray (Feb-Jun)	Experimental (5269)	738	57	7.72	5.69	14.00	10.81
		Comparison (3530)	539	138	25.60	17.99	15.26	39.09
	Post-spray after IV round (Aug-Oct)	Experimental (5269)	556	40	7.20	4.50	10.55	7.59
		Comparison (3530)	371	91	24.52	19.67	10.50	25.77
	After V round (Dec 88-Mar 89)	Experimental (5269)	539	23	4.26	3.15	10.22	4.36
		Comparison (3530)	538	94	17.47	13.38	15.24	26.62

BS — Blood smear; SPR — Slide positivity rate; SfR — Slide falciparum rate; BER — Blood examination rate, and PI — Parasite incidence.

Susceptibility tests

Susceptibility tests carried out before deltamethrin spray revealed that *An. culicifacies*, *Cx. vishnui* and *Cx. quinquefasciatus* mosquitoes were highly susceptible to deltamethrin, but resistant to DDT and HCH. No change was observed in the susceptibility status of these mosquitoes against deltamethrin, after three years of deltamethrin spray. Thus, the results of susceptibility test carried out against above mosquitoes with deltamethrin after three years of deltamethrin spray indicate that there was no selection of resistance in any of these species against this insecticides. But, development of high degree of resistance in *Cx. quinquefasciatus* against deltamethrin was reported during a field trial which could not be confirmed during the present study[12].

Toxicological evaluation

Effect on human/animal population and acceptability: No ill effect of deltamethrin spray was observed on the inhabitants, spray personnels and animal population of the area during and after the spray, except slight eye and skin irritation, experienced by the workers who did not use protective coverings and eye glasses during the spray. Such irritation disappeared after thorough washing with soap.

It was noticed that deltamethrin spray had a very high acceptability by the inhabitants of the area, as evident by their response in the form of willingness to get their houses sprayed with new insecticide.

Other collateral benefits

These benefits include total disappearance of various other house hold pests, viz. house flies, bed bugs, crickets and other crawling insects. The spraying did not spoil the wall surface as the spray impression was very poorly visible. There was no complaint of any odour or other undesirable characteristics.

Thus, it is evident from the above observations that deltamethrin spray showed no adverse effect on the inhabitants, spraymen, animal population and other non-target organisms of the area. These characteristics would make this insecticide popular and readily acceptable to the general public during its use in various public health programmes. Similar type of response for deltamethrin spray was noticed by other workers[11].

Parasitological evaluation

The data on the parasitological indices in experimental and comparison areas, before and after each round of spray is summarised in Table 2. It may be noted that before the beginning of the field trial the parasite incidence (PI) in experimental area was about two times higher than the comparison area. More than 80 to 90 per cent of malaria cases were due to *P. falciparum* infection in both

the areas. Similarly, SPR of experimental area was 35 per cent more than the comparison area before the spray.

The impact of deltamethrin spray on malaria cases was monitored by active surveillance method in the selected villages of both the areas. From the table, it may be seen that in the experimental area, there was a decline in SPR from 17.96% in 1986 to 4.26% in 1988-89, thus showing a reduction of 76%. On the contrary, SPR in the comparison area showed considerable increase from 10.09 to 17.47 per cent. Similarly, slide falciparum rate (SfR) at the end of the V round of deltamethrin spray showed a reduction of 81% in experimental area. On the other hand in comparison area, SfR values showed an increase of 42 per cent. The parasite incidence (PI) in experimental area declined from 20.87 in 1986 to 4.36 in 1988-89, thus showing a reduction of 78 per cent, whereas, in comparison area, the PI showed an increase from 10.13 to 26.62 during the same period, thus showing an increase of 163 per cent.

It is discernible from the above data that there is a considerable reduction of malaria cases in the experimental area at the end of the V rounds of deltamethrin spray. On the other hand in comparison area, which was sprayed with HCH, malaria cases had increased. The incidence of *P. falciparum* cases in comparison area increased, whereas, the area sprayed with deltamethrin showed almost consistent decrease in *P. falciparum* incidence. Such drastic reduction in malaria incidence could be attributed due to reduction in active malaria transmission because of the low vector density after deltamethrin spray.

Similar reduction in the number of malaria cases was reported during the village scale and group of villages scale field trial in Ghaziabad district (U.P.)[11,12].

In view of the above parasitological and entomological data, it may be said that malaria incidence in a highly endemic areas of Jagdalpur and other such areas of India can be brought down by applying two rounds of deltamethrin spray annually at a dosage of 25 mg/sq m.

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Recent Epidemiological Status of Malaria in Calcutta Municipal Corporation Area, West Bengal

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In Calcutta, *Plasmodium falciparum* cases and death due to malaria show remarkably increasing trend since 1990. The incidence of *P. falciparum* malaria has increased more than eleven folds in 1996 in comparison to 1990, with 0, 0, 0, 3, 7, 52 and 17 deaths in 1990, 1991, 1992, 1993, 1994, 1995 and 1996 respectively. Situation is more serious than what it is projected in official records as annual blood examination rate (ABER) in Calcutta Municipal area is poor, varying from 1.5 to 3.9 in 1993 and 1996 respectively.

This is further evident from the fact that in a study area in 19 months (January 1995 to July 1996) the slide positivity rate (SPR) was 47.94% on an average 28.72% suffer from *P. falciparum* infection (as low as 0.5% in June 1996 and as high as 71.5% in November 1996). For the first time resistance of *P. falciparum* to chloroquine has been noted at RII and RIII level. The response of the same parasite strain to sulfa-pyrimethamine combination drug is very much promising. Fresh infection is occurring in all the months of the year and the favourable period is from July to November 1995 that is corroborating with Container index and Breteau index related to the vector mosquito *Anopheles*. Susceptibility status of *An. stephensi* indicates that the mosquito species has acquired resistance to DDT, BHC, propoxure and malathion but is still susceptible to fenthion and deltamethrin.

Keywords: Calcutta, Epidemiology, Malaria

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INTRODUCTION

Malaria is exclusively a local and focal phenomenon. The intensity of transmission, prevalence and distribution of parasites is determined by local malariogenic conditions which differ from village-to-village, city-to-city and even in some localities varying from ward-to-ward.

Although, the urban malaria scheme had been introduced into National Malaria Eradication Programme in 1971[1], its function was reviewed in depth during the introduction of Modified Plan of Operation in 1972[2]. The expert committee in 1995 identified 15 major cities including the area under Calcutta Municipal Corporation (CMC) where malaria situation was serious. As per state National Malaria Eradication Programme report, the areas under CMC alone contributed 40.2% of the total malaria cases and 59.7% confirmed death due to malaria in West Bengal in 1995.

In order to assess actual situation of malaria in the city of Calcutta, a longitudinal study was conducted in a highly malaria affected ward to note the slide positivity rate (SPR), *Plasmodium falciparum* infection and its susceptibility status to antimalarials. An entomological survey, along with susceptibility status of vector (*Anopheles stephensi*) to different insecticides was also done.

MATERIALS AND METHODS

The city of Calcutta that was first established by the English "East India Company of Merchants and Traders" is 1690 in situated at the longitude of 88° 20'E and latitude of 22° 82'N and 5.3 m above the sea level. Today, as per 1991 census that area of Calcutta Municipal Corporation is 187.33 sq km having a population of 4.38 million, with the population density to the tune of 23,670 inhabitants per sq km[3].

The detailed data on malaria diagnosed and treated cases is different hospitals, Government undertaking Hospitals, clinics of CMC and state Government were collected since 1990 to September 1997 that was verified with the malaria data maintained by state NMEP. The data were analysed using standard epidemiological parameters. A ward No. 84, Monoharpukur area consisting of 20,000 population in south Calcutta was selected for the study area because large number of malaria cases, specially *P. falciparum* were reported in this ward. The study was carried out from January 1995 to July 1996 in the following events.

Monthly house-to-house fever survey was done between January 1995 and July 1996. Thick and thin blood smears were collected from all fever cases. The slides were stained by JSB stain and examined for malaria parasites. All the patients were treated with antimalarials as recommended by Malaria Action Programme (MAP) in 1995[2].

Sensitivity status of *P. falciparum* was evaluated following WHO *in vivo* technique[4]. Simplified *in vivo* test was conducted administrating 600 mg (adult dose) chloroquine to *P. falciparum* ring positive patients and slides

from individual patients were collected on D 0, D 2 and D 7. Standard *in vivo* (7-days) test was conducted with 1500 mg (adult dose) chloroquine or 2 tablets (adult dose) of sulfadoxin-pyrimethamine combination drug (1000 mg sulfa + 50 mg pyrimethamine). Blood smears were collected from each study patient daily from D 0 to D 7. Smears were stained with Giemsa and examined and parasite count was made in thick smears against 1000 WBCs. Response of malaria parasite to antimalarial drugs was assessed following WHO criteria[4].

Monthly larval breeding spots were searched in fixed hundred houses. Water reservoirs showing breeding of mosquitoes were noted. Larvae were collected and identified by standard entomological technique[5,6] to determine the Container index and Breteau index of *Anopheles stephensi*. The data was compared with monthly malaria data collected from the study area to note if there was any correlation with the indices and number cases per months.

Wild *An. stephensi* larvae were reared to adult in the laboratory. Twenty or twenty-five laboratory bred blood fed healthy females were exposed to DDT, fenthion, fenitrothion, propoxure and deltamethrin (diagnostic dose) impregnated papers following usual WHO technique[7]. At least four replicates with controls were tested against each insecticide, maintaining temperature at 24 $\pm$ 2°C and relative humidity of 65 $\pm$ 5%. Twenty, III or early IV instar larvae were exposed to diagnostic dose of larvicides like malathion, fenthion and fenitrothion following standard WHO technique[7]. Minimum four replicates against each larvicides were run along with control. The tests were conducted between December 1995 and January 1996.

RESULTS AND DISCUSSION

Table 1 shows the epidemiological situation of malaria in West Bengal and CMC area. The data show that the incidence of malaria including *P. falciparum* case and death due to malaria has remarkable increasing trend since 1990. The annual blood examination rate (ABER) as reported in CMC area is extremely poor that varies between 1.5 to 3.9 in 1993 and 1996 respectively. In spite of poor ABER, high slide positivity rate (SPR) in CMC area was found to vary between 17.7 to 28.1 in 1991 and 1995 respectively. The incidence of malaria has increased nearly 3 folds in 1996 (total positive cases were 44,602) as compared to 1990 (13,624 cases). The incidence of *P. falciparum* also has increased more than eleven folds in 1996 (6467 cases) as compared to 1990 (552 cases). CMC is also recording the increased number of death due to malaria during past four years. The deaths due to malaria were recorded as 3, 7, 52 and 17 in 1992, 1993, 1994 and 1996 respectively.

Table 2 shows the malariological data in the study area from January 1995 to July 1996. Within these 19 months of study, a total of

Table 1. Epidemiological data on malaria in West Bengal and Calcutta Municipal Corporation area

Year	Blood slide examined	Total (+) ve	*Pf* cases	Deaths	ABER	*Pf* %	SPR
West Bengal							
1990	1610243	27531	3690	4	2.79	13.4	1.71
1991	1345197	40452	7771	13	3.19	19.2	2.17
1992	2159242	28179	7858	43	3.73	27.8	1.31
1993	2078000	46138	8723	37	3.40	18.9	2.2
1994	2535647	74293	15379	52	4.10	20.5	2.9
1995	2528000	91014	17226	87	4.0	18.9	3.6
1996	2172983	80127	15629	55	–	17.0	3.7
1997*	1302740	38795	4173	22			
Calcutta							
1990	76936	13624	552	0	2.0	0.7	17.7
1991	66854	15606	708	0	1.8	1.1	23.3
1992	82180	17969	728	0	2.2	0.9	21.8
1993	74161	19084	1802	3	1.5	2.4	25.6
1994	85815	18298	2283	7	2.2	2.7	21.3
1995	131000	36804	5725	52	2.9	4.4	28.1
1996	174000	44602	6467	17	3.9	14.5	25.6
1997*	77994	15719	1084	14			

*Provisional data up to September 1997.

8432 blood slides has been collected of which 4045 were found positive for malaria. The SPR is calculated to be 47.97%. Incidence of malaria was noted throughout the year in Calcutta. The highest SPR (69.4%) was recorded in the month of February 1995 and the lowest (27.5%) in the month of February 1996. This decline in SPR, is perhaps, due to constant surveillance and treatment and mass awareness among the local inhabitants regarding malaria transmission. Average 28.72% *P. falciparum* infection has been noted in the study period that is as low as 0.5% (June 1995) and as high as 71.5% (November 1996). Malaria cases along with *P. falciparum* were found to occur throughout the study period of 1995-96. The same types of findings have been noted by Hati[8] based on available Government data from 1981 to 1988. The maximum numbers of cases have

Table 2. Malariological data of study area of Monoharpukur, Calcutta (1995 and 1996)

Year/Month		Total slide examined	Total (+)ve	SPR	Total *Pf*	*Pf*%	Container index	Breteau index
1995	Jan	140	61	43.5	41	67.2	10	10
	Feb	108	75	69.4	34	45.3	12.6	12
	Mar	131	89	67.9	35	39.3	13	11
	Apr	153	94	61.4	12	12.7	10	10
	May	248	159	64.1	3	1.8	8.2	8
	Jun	322	184	57.1	1	0.5	8.8	8
	Jul	533	307	57.5	35	9.7	10	9
	Aug	1129	531	47.0	71	13.3	7.8	8
	Sep	778	381	48.9	14	3.6	9.2	9
	Oct	750	392	52.2	110	28.0	12.5	12
	Nov	1225	630	51.4	451	71.5	14	12
	Dec	776	301	38.8	248	82.3	12.2	12
1996	Jan	330	107	32.4	54	50.4	2	2
	Feb	218	60	27.5	27	45.0	2.2	2
	Mar	96	32	33.3	1	3.1	7.5	5
	Apr	178	72	40.4	1	1.3	10	8
	May	252	123	48.8	5	4.0	12.2	9
	Jun	318	127	39.9	8	6.2	10.9	10
	Jul	747	320	42.8	11	3.4	12.5	12
Total		8432	4045	47.97	1162	28.72		

been recorded in the months of July to November 1995 that corroborates with the data of Hati *et al*[9].

Seventeen *P. falciparum* cases were studied giving 600 mg chloroquine single (adult) dose. Results of these studies show that one case each (5.88%) at RII and RIII level of resistance. Forty-two cases were studied against 1500 mg chloroquine (adult dose) given over three days out of which only one case (3.38%) showed resistance to chloroquine at RIII level. Study of 23 cases with sulfa-pyrimethamine showed satisfactory response (S/RI) (Table 3). It is clear from the present study that *P. falciparum* resistant to chloroquine has certainly started emerging out in the city and recorded scientifically for the first time. Although the numbers of resistant cases are very low yet it is of great concern

as the resistance is of higher degree. The low grade resistance (RI) could not be identified as the study was limited to 7-days, observations where delayed recrudescence of RI cases remained left out.

It is also seen that the dose of chloroquine does not play any significant role either in the level of resistance or in its number. To greater satisfaction, it has been observed that, the response of other antimalarial drug, sulfapyrimethamine to the same parasite strain of *P. falciparum* is promising. The real magnitude of the phenomenon can only be established with a wider exploration, i.e. including greater area and large number of cases in the study.

Table 2 also shows the Container index and Breteau index of *An. stephensi* are found to breed in a wide range of water collections throughout the year. The maximum breeding, i.e. Container index was found in rainy season, i.e. in month of July to October clearly indicating that the index rises with the rise in incidence of malaria. This finding also corroborates with the findings of Hati *et al*[9]

The results of susceptibility status of *An. stephensi* adults against DDT (4%), fenthion (1%), fenitrothion (1%), propoxure (0.1%) and deltamethrin (0.025%) are shown in Table 4. The percentage mortality of the mosquito species in one hour exposure against DDT, fenthion, fenitrothion and propoxure was noted as 55, 100, 83.3 and 53.3% respectively. Hati *et al*[10], noted resistance to both DDT and dieldrin amongst *An. stephensi* in Calcutta. LC_{50} value of *An. stephensi* was calculated by them as 1.36% to DDT. In the

Table 3. Sensitivity status of *Plasmodium falciparum* to antimalarials in study area under Calcutta Municipal Corporation

Period	Drugs/dose (adult)	Test sample	Status of response	Average parasite count per c.mm of blood on follow-up days							
				Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Dec 94	Chloroquine (600 mg)	15	S/RI	1293	–	10	–	–	–	–	0
Jan 95	-do-	1	RII	1981	–	121	–	–	–	–	288
-do-	-do-	1	RIII	1102	–	387	–	–	–	–	58
Dec 95	Chloroquine (1500 mg)	41	S/RI	1556	443	97	8	10	0	0	0
-do-	-do-	1	RIII	4797	652	1308	901	140	36	2	33
Jan 96	Sulpha-pyri-methamine (2 tabs)	23	S	1668	348	18	0.5	0.2	0	0	0

present study, 55% mortality of the mosquito species was noted during December 1995 to January 1996 in one hour exposure to DDT, confirming that the species was still resistant to DDT, although DDT was not being sprayed in Calcutta as part of Urban Malaria Programme since 1971. No base line data are available on sensitivity status of the species to organophosphorus and synthetic pyrethroids in Calcutta. The area under Calcutta Municipal Corporation is under the pressure of different insecticides like Finite (fenitrothion), Baytex (fenthion), Abate (temephos), Baygon concentrate and Baygon bait (propoxure), Hexit (deltamethrin) used indiscriminately by various private Pest Control Organizations and residents of Calcutta. The present study has revealed that *An. stephensi* adults have developed resistance to propoxure too but are still susceptible to fenthion and deltamethrin.

Susceptibility status of *An. stephensi* larvae to malathion (3.125 ppm), fenthion (0.125 ppm) and fenetrothion (0.125 ppm) presented in Table 5 shows 61.6, 100 and 96.6% mortality of larvae against malathion, fenthion and fenitrothion respectively indicating that *An. stephensi* larvae of Calcutta have developed resistance to malathion.

Table 4. Susceptibility status of *Anopheles stephensi* adult to insecticides in selected areas of Calcutta in 1995

Insecticide	Concentration of insecticides Exposure x Time (min)	No. exposed	No of death after 24 h	% mortality
DDT	4.0% x 60	60	33	55
Control	– x 60	60	00	00
Fenitrothion	1.0% x 60	60	50	83.3
Control	– x 60	60	00	00
Fenthion	1.0% x 60	50	50	100
Control	x 60	50	01	2
Propoxure	0.1% x 60	60	32	53.3
Control	– x 60	60	02	3.3
Deltamethrin	0.125% x 15	40	36	90
Deltamethrin	0.125% x 30	60	60	100
Control	– x 30	50	00	00

Table 5. Susceptibility status of *Anopheles stephensi* larvae to organophosphorus insecticides in selected areas of Calcutta in 1995

Insecticide	Concentration (mg/l)	No. exposed	No. of death	% mortality
Malathion	3.125	60	37	61.6
Control	–	60	0	00.0
Fenthion	0.125	100	100	100
Control	–	60	0	00.0
Fenitrothion	0.125	60	58	96.6
Control	–	60	0	00.0

Therefore, both mortality and morbidity due to malaria in the area of Calcutta Municipal Corporation showed increasing trend. In this study area, the average SPR per cent was noted as 47.97% between January 1995 to July 1996 with 28.7% *P. falciparum* infection. For the first time, development of chloroquine resistance was detected against *P. falciparum* in the city of Calcutta. The vector *An. stephensi* also developed multiple resistance against the insecticides like DDT, malathion and fenitrothion.

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Observations on Mosquito Breeding in Rice Fields in Two Ecological Terrains of District Jabalpur, Madhya Pradesh

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Studies on ecological succession of anophelines and the malaria vectors were carried out in rice fields located in two ecologically different terrains, i.e. plains and forested hills in Jabalpur district during monsoon (July-October 1995). Nine and fifteen species of anophelines were found breeding in the rice fields of plain and forested hill villages respectively. *Anopheles culicifacies* and *An. subpictus* were dominant species in both the areas. The percentage emergence of adults of *An. annularis, An. nigerrimus* and *An. pallidus* was higher in plain villages while the percentage of *An. theobaldi, An. fluviatilis* and *An. jeyporiensis* was higher in forested hill villages. Among malaria vectors, three species were found breeding in rice fields of hilly terrain, i.e. *An. culicifacies, An. fluviatilis* and *An. stephensi* whereas in rice fields of plains *An. culicifacies* and *An. fluviatilis* were recorded.

Keywords: Breeding, Jabalpur, Mosquito, Ricefield

INTRODUCTION

Rice forms the staple diet of about 80 per cent of the world's population. In India about 41.66 million ha land is utilized for rice cultivation, of which 5 million ha (12%) is utilized in Madhya Pradesh[1]. In Jabalpur district 15 per cent of the population utilize rice which is grown in 0.15 million ha land[2]. It is well known that rice fields are major source of anopheline breeding[3,4]. The association of anopheline breeding and location of rice fields within villages was recorded in Mandla district (M.P.)[1] No information about mosquito breeding in rice fields was available from other rice growing areas of M.P. There-

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fore, a study was undertaken to investigate mosquito breeding, emergence, relationship with different stages of rice growth and contribution of rice fields in production of anopheline vectors in rice fields located in two ecologically different terrains, i.e. plain and forested hill of District Jabalpur.

MATERIALS AND METHODS

Rice field situated in two ecologically different terrains, viz. plains and undulating forested hills in Jablapur were selected. The study was carried out during July to October 1995, the main rainy season in this part of the country, in four villages, two from plains and two from forested hills. The distance between the two group of villages was about 15 km. In each village four rice fields at a distance of 500 m from the village were chosen. The main indigenous varieties grown by the farmers in both the areas were *Nungi, Batro, Luchai, Ratna* etc., which harvested in about 120 days. Fishes, bugs, insects etc. were found in all the rice fields under the study.

Rice fields in the plains were situated near main Bargi irrigation canal. Soil was red, brown and sandy. Although the villages had a network of minor canals yet the rice cultivation was mainly monsoon dependent.

In the forested hills the study villages were inhabited by tribals and were located amid patches of mixed forests, i.e. Teak (*Tectona grandis*), Mahua (*Madhuca indica*) and Sal (*Shorea robusta*). No canal system existed but there were perennial streams with rocky pools, pits and seepages. The rice fields selected for the study were situated on the stream banks. The crops were flooded with water which was allowed to stand continuously for months. The soil was laterite.

Prior to the commencement of monsoon, rice is generally sown in a nursery in standing water and then seedlings are transplanted in submerged fields. After the rain, algal growth invariably covered the water surface. Since rice cultivation is monsoon dependent, the fields remained dry for a few days between spells of rainfall thereby creating innumerable puddles. Weekly sampling of the mosquito immatures was carried out in each rice field since rice transplantation in July and observations were made until the harvest in October. Collections of immatures were restricted to only 10 dips from each field using a 250 ml larval dipper. Simultaneously the height of the rice plants was measured from each rice field by a measuring scale and water temperature of each rice field was recorded.

The larvae and pupae collected, were brought to the laboratory, counted and allowed to emerge to the adult stage. Indoor resting man hour density was monitored monthly from each of the four index villages using standard entomological technique[5]. The adults were identified with the keys of Christophers[6] and Barraud[7]. Correlation between height of rice plants and proportion of emer-

gence of most abundant species was tested by Pearson 'r' correlation coefficient test.

RESULTS

Results of larval density and emergence for both plain and forested hill villages are shown in Table 1. The average larval density of anophelines per dip was higher (3.08) in the rice fields in forested hills than in those in the plains (2.23). The larval density of anophelines was highest in August in rice fields of both type of villages and thereafter it showed a gradual decline. The average larval density of culicines was very low (< 1.1 per dip) during the entire period.

In rice fields of plain villages, nine anopheline species, viz. *Anopheles culicifacies*, *An. subpictus*, *An. fluviatilis*, *An. annularis*, *An. theobaldi*, *An. jeyporiensis*, *An. vagus*, *An. pallidus* and *An. nigerrimus* emerged. In addition to these species, six more species, viz. *An. stephensi*, *An. splendidus*, *An. barbirostris*, *An. varuna*, *An. aconitus* and *An. maculatus* emerged from rice fields in the hills. *An. culicifacies* was the most abundant species in both the terrains but its proportion in total emergence of all species was higher (52.4%) in rice fields in forested hills than in those in the plains (42.7%). Among other anopheline species found in both type of villages, the proportion of *An. subpictus*, *An. annularis* and *An. nigerrimus* was higher in the plains (47.9, 4.3 and 1.8% respectively) in comparison to forested hills (36.0, 0.6 and 0.7% respectively). The proportion of *An. fluviatilis*, *An. theobaldi* and *An. jeyporiensis* was higher in forested hill than plain villages. It was 1.8, 4.0 and 0.9% respectively in forested hill and 0.5, 0.1 and 0.3% respectively in plain villages. Two species, *An. vagus* and *An. pallidus* were found almost in equal proportions in both type of villages.

In addition culicine species, viz. *Culex sinensis*, *Cx. vishnui*, *Cx. bitaeniorhynchus*, *Cx. tritaeniorhynchus*, *Cx. mimulus* and *Aedes vittatus* were commonly recorded from both type of villages, while *Cx. quinquefasciatus* and *Cx. tenuipalpis* were not recorded from rice fields of forested hill villages and *Cx. fuscanus* was not found in rice fields of plains. Only *Cx. sinensis* and *Cx. vishnui* were the most prevalent species among culicines in both type of villages.

Table 2 shows data on the proportion of different anophelines recorded in relation to the plant growth. Results revealed that *An. culicifacies* was the most abundant species in the early stages of rice plants. The heavy breeding in this stage was encountered in shallow pools and pits with and without vegetation. Thereafter the density decreased gradually. The percentage of declining trend from July to October was 54.5 to 17.6 in plain and 70.1 to 28.1 in forested hill villages respectively. The proportion of emergence of *An. culicifacies* with plant height showed a negative correlation (–0.90676 Pearson 'r' value) in both the plains and forested hill villages.

Table 1. Results of rice field breeding survey

Larval density/ dip	Villages in plains					Villages in forested hills				
	Jul	Aug	Sep	Oct	Av.	Jul	Aug	Sep	Oct	Av.
Anopheline	1.90	4.26	1.59	1.16	2.23	1.56	5.55	4.40	0.80	3.08
Culicine	0.12	0.75	1.08	0.44	0.60	0.30	0.73	0.76	0.30	0.52
Species emerged (%)										
Anophelines										
An. culicifacies	66.20	40.00	26.80	18.90	42.70	71.40	58.80	36.30	30.00	52.40
An. subpictus	32.00	55.20	52.20	29.70	47.90	25.80	35.70	40.50	38.50	36.00
An. fluviatilis	0.60	0.50	0.70	0.00	0.50	0.90	1.70	2.10	3.80	1.80
An. annularis	0.60	2.90	8.90	18.90	4.30	1.80	0.00	1.10	0.00	0.60
An. theobaldi	0.00	0.00	0.70	0.00	0.10	0.00	0.60	11.00	3.80	4.00
An. jeyporiensis	0.00	0.003	0.70	0.00	0.30	0.00	1.50	0.30	0.00	0.90
An. vagus	0.00	0.50	2.90	5.40	1.10	0.00	1.10	1.10	3.80	1.00
An. pallidus	0.60	0.50	3.00	5.40	1.20	0.00	0.00	2.80	3.80	1.00
An. nigerrimus	0.00	0.00	3.70	21.60	1.80	0.00	0.00	0.70	15.40	0.70
Culicines										
Cx. sinensis	36.40	42.20	43.70	42.80	42.40	32.30	32.50	55.00	37.00	38.80
Cx. vishnui	0.00	32.00	41.60	38.10	33.80	41.10	50.60	35.30	52.10	45.70
Cx. bitaeniorhynchus	0.00	14.40	0.00	0.00	7.10	0.00	3.60	0.00	0.00	1.60
Cx. tritaeniorhynchus	0.00	7.20	14.60	17.60	11.10	0.00	12.00	0.00	4.30	5.60
Cx. mimulus	18.20	0.00	0.00	0.00	1.00	8.80	0.00	9.80	6.50	5.10
Total anopheline	169	382	134	37	722	112	457	281	26	876
Total culicine	11	97	48	42	198	34	83	51	46	214

An. stephensi, An. splendidus, An. barbirostris, An. varuna, An. aconitus, An. maculatus, Cx. quinquefasciatus, Cx. tenuipalpis, Cx. fuscanus and *Ae. vittatus* emerged in low numbers only.

Table 2. Relationship of plant height and anopheline species breeding in the rice fields

Species	Height of plants (cms)											
	0-20		21-40		41-60		61-80		81-100		>100	
	Plains	Hills	Plains	Hills	Plains	Hills	Plains	Hills	Plains	Hills	Plains	Hills*
An. culicifacies	54.5	70.1	46.0	60.2	41.2	56.8	23.6	37.2	21.4	28.1	17.6	
An. subpictus	44.0	25.3	52.3	37.0	45.6	34.3	60.5	39.4	35.7	46.4	23.5	
An. fluviatilis	0.5	1.5	0.0	0.7	1.5	2.9	0.0	1.7	2.3	5.6	0.0	
An. annularis	0.5	3.0	1.3	0.0	7.3	0.0	6.6	1.3	21.4	0.0	28.5	
An. theobaldi	0.0	0.0	0.0	0.3	0.7	0.9	0.0	12.1	0.0	5.6	0.0	
An. jeyporiensis	0.0	0.0	0.0	0.7	0.7	2.4	0.0	0.4	0.0	0.0	0.0	
An. vagus	0.0	0.0	0.4	0.7	0.7	0.9	3.9	1.7	4.7	1.4	5.9	
An. pallidus	0.5	0.0	0.0	0.0	2.2	0.9	2.6	2.1	2.4	2.8	11.7	
An. nigerrimus	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.4	11.9	5.6	2.72	

*Rice fields became dry; *An. stephensi, An. splendidus, An. barbirostris, An. varuna, An. aconitus* and *An. maculatus*, emerged from rice fields situated in forested hill villages in small numbers.

Breeding of *An. subpictus* occurred since beginning and continued up to mature rice plants in both the plain and forested hill villages (Table 2). Most rice fields in which *An. fluviatilis* breeding was found had a perceptible flow of water. The emergence of this species was maximum (2.3% in plains and 5.6% in forested hills) when the plant height was between 81 and 100 cms.

Table 3 shows data on man hour densities of anopheline species for four months, monitored from the fixed catching stations in the index villages. Only nine species were recorded, of which *An. culicifacies, An. subpictus* and *An. annularis* were prevalent during entire period of study in both type of villages. However, the density of *An. culicifacies* was significantly higher in rice fields of forested hill villages (64.5) than the rice fields of plain villages (36.7, paired 't' test $p \leq 0.0006$).

DISCUSSION

In consistence with earlier results[1,8,9] larval density of *An. culicifacies* in rice fields showed gradual decline in later stages of rice plants which might be due to mechanical obstruction for oviposition due to vigorous vegetative growth of paddy plants[4]. Similarly, overall anopheline larval density in both type of villages showed decline in September-October which might be due to non-availability of water during later stages of rice plants towards the end of monsoon

Table 3. Per man hour density of anophelines collected from villages near rice fields

Species	Villages in plains					Villages in forested hills				
	Jul	Aug	Sep	Oct	Av.	Jul	Aug	Sep	Oct	Av.
All Anopheles	60.5	103.5	81.0	42.5	71.9	65.0	129.5	93.5	98.5	96.6
An. culicifacies	32.0	57.0	29.0	29.0	36.7	45.0	86.0	63.5	63.5	64.5
An. subpictus	26.0	42.0	43.0	8.0	29.7	11.0	39.5	22.5	25.0	24.5
An. annularis	2.5	3.5	9.0	3.0	4.5	9.0	2.5	7.0	10.0	7.1
An. fluviatilis	0.0	0.0	0.0	1.0	0.2	0.0	1.0	0.0	0.0	0.2

An. pallidus, *An. nigerrimus* and *An. theobaldi* in plain villages while *An. varuna* and *An. vagus* in forested hill villages were recorded in small numbers.

period. In forested hill villages *An. culicifacies* succeeded mainly by breeding of *An. fluviatilis*, *An. theobaldi*, *An. splendidus* and *An. nigerrimus* where as in plain villages it was took over mainly by *An. annularis* and *An. nigerrimus*. In this study *An. theobaldi* and *An. nigerrimus* breeding increased in later stages of paddy growth as recorded earlier[1]. Water temperature in the rice fields was between 26 to 33°C during the study, which is ideal for the growth of egg, larvae and pupae of anophelines[10]. Data on month-wise man hour density of anophelines and larval density (per dip) in rice fields of both terrain showed that there were apparently some relationship as the two increased or decreased together.

The larval density of anophelines and emergence of *An. culicifacies* and *An. fluviatilis* were higher in rice fields of villages in forested hills and simultaneously, a few specimen of another vector *An. stephensi* were also found breeding in these villages. Thus it is clear that rice fields situated in forested hill villages are more responsible for vector propagation by contributing high density of *An. culicifacies* in initial stage followed by *An. fluviatilis* and *An. stephensi* in later stages of succession.

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SHORT NOTES

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A Report of Mosquito Fauna Survey and Vector Incrimination in Goalpara District of Assam

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Keywords: *Anopheles minimus*, Assam, Malaria vector

In Assam, first survey for anophelines was carried out in the year 1923[1]. After that, several such studies were carried out in various places of the state[2,3] and the role of *Anopheles minimus* in transmitting malaria was established. Subsequent studies revealed the disappearance of *An. minimus*[4-6] in some areas which has been attributed to the large scale use of DDT under National Malaria Eradication Programme (NMEP) and other pesticides used in agriculture. In Goalpara, which is an old district of Assam situated at its western corner, no study on mosquitoes was carried out except one report of vector incrimination from the adjacent Kokrajhar district (earlier in Goalpara) way back in 1936[7]. Goalpara district is important for high incidence of malaria with API (Annual Parasite Index) ranging from 3.28 to 17.95 during recent years (Table 1). An entomological study was conducted in the Agia PHC area of this district which experienced a malaria epidemic during study year 1995 showing SPR (Slide Positivity Rate) and API values of 13.0 and 10.2 respectively. This PHC consists of 2,68,194 population among which a large population of Garo tribes inhabit mostly in the foothill areas bordering Meghalaya. A faunis-

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Table 1. Record of malaria cases in Goalpara district of Assam during 1993-95

Year	Population	BSE	(+)ve	*Pf*	*Pv*	Deaths	API
1993	769000	96657	2523	2284	239	0	3.28
1994	781000	64181	3569	3373	196	0	4.56
1995	797000	107287	14311	12502	2009	9	17.95

Source: NMEP.

Table 2. Record of collection of mosquitoes in Agia PHC area of Goalpara district, Assam

Species	By operating CDC light-trap (1700-0500 hrs)			By suction tube indoor during morning hours	
	Total No./trap night		%	Total	MHD
An. annularis	920	460.0	26.87	3	0.75
An. dirus	6	3.0	0.17	–	–
An. jeyporiensis	54	27.0	1.57	–	–
An. karwari	216	108.0	6.30	–	–
An. kochi	212	106.0	6.19	–	–
An. majidi	2	1.0	0.06	–	–
An. minimus	118	59.0	3.44	13	3.25
An. peditaeniatus	53	26.5	1.55	–	–
An. splendidus	28	14.0	0.82	–	–
An. tessellatus	57	28.5	1.66	–	–
An. jamesii	9	4.5	0.26	–	–
An. vagus	70	35.0	2.04	35	8.75
Cx. fuscocephala	124	62.0	3.62	–	–
Cx. gelidus	60	30.0	1.75	–	–
Cx. pseudovishnui	221	110.5	6.45	–	–
Cx. vishnui	1176	588.0	34.34	3	0.75
Cx. quinquefasciatus	–	–	–	40	10.00
Ma. annulifera	19	9.5	0.55	–	–
Ma. uniformis	30	15.0	0.87	–	–
Ae. lineatopennis	9	4.5	0.26	–	–
Ar. kuchingensis	40	20.0	1.16	–	–
Total	3424	1712.0	100.00	94	24.00

tic study was taken up in two tribal villages during the first week of November 1995. Mosquitoes were collected using CDC miniature light-traps for whole nights and in the morning using suction tube in houses. The collected mosquitoes were transported to the field laboratory for identification of species and for vector incrimination.

Table 2 shows the record of mosquitoes collected from the study area. In light-traps operated for two nights (one trap during each night placed in cattlesheds adjacent to human dwellings), a total number of 3424 mosquitoes of 21 different species were collected. The genus *Anopheles* consisted of 12 species of which *An. annularis* was the predominant (26.8%) followed by *An. karwari* (6.3%) and *An. kochi* (6.2%). Among the culicines, five species of *Culex*, two species of *Mansonia* and one species each of *Aedes* and *Armegeres* were collected. *Cx. vishnui* was the predominant species forming 34.4% of the total collection. In indoor survey conducted in the morning over four man hours, only five species of mosquitoes were collected among which *Cx. quinquefasciatus* was the most common species (MHD 10.00) followed by *An. vagus* (MHD 8.75) and *An. minimus* (MHD 3.25).

The results of dissection of *Anopheles* species (potential vectors of malaria) collected from all sources revealed the presence of natural infection of malaria in two specimens of *An. minimus* showing sporozoite rate of 1.6 per cent for that period. *An. dirus* was also recorded in light-trap collection but its density was low (3 per trap). In pre- DDT era, *An. minimus* was considered to be the major vector of malaria in Assam and other northeastern states of India. However, after commencement of DDT spraying in 1958 under NMEP, this species showed transient disappearance and was thought to have disappeared from the northeastern region of India[4-6]. But in recent studies, *An. minimus* has again been incriminated from various places of Assam[8-10]. The present study confirms the role of *An. minimus* as a vector of malaria in foothill areas of lower Assam.

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Larvicidal Efficacy of *Bacillus sphaericus* H-5a, 5b and *B. thuringiensis* var. *israelensis* H-14 against Malaria Vectors in Bhabar Area, District Naini Tal, U.P.

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Keywords: Biolarvicides, Malaria vectors

Development of insecticide resistance in vectors species and their high cost made it apparent to search for alternate vector control methodology. Biological control methods such as biolarvicides has received additional impetus for control of vectors. *Bacillus thuringiensis* serotype H-14 has been shown to be highly effective against mosquito larvae and black fly vectors and also reported safe for non-target organism[1]. *Bacillus sphaericus* was reported more effective than *B. thuringiensis* var. *israelensis* especially against culicine larvae, its residual efficacy persisted for several weeks even in polluted waters[2]. To know the efficacy of two Russian origin biolarvicides, i.e. *Bacillus sphaericus* H-5a, 5b (Spherix) and *Bacillus thuringiensis* var. *israelensis* H-14 (Bactoculicide) in cemented tanks and domestic ponds in Bhabar area of District Naini Tal, applications were made during the month of October 1993 and larvicidal effect against vector anophelines were assessed. During this period the mean temperature was 21.6°C and relative humidity was 65 per cent. The results of the studies are mentioned in this communication.

Below the foothill of Himalaya lies Bhabar which constitutes 10-12 km wide belt of for-

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est area. It is a dry upland with low water table and sandy loam soil area. Water in this area is supplied from Gola River at weekly/ fortnightly interval by canal system. The water is stored in underground cemented tanks for drinking purpose and in domestic ponds for the animals. Tanks and domestic ponds are thus available in and around each house which are potential mosquito breeding sites and support breeding of malaria vectors, i.e. *An. culicifacies* and *An. fluviatilis*[3]. A total of 14 cemented tanks and 10 small ponds of various size measuring from 2.7 to 16.8 sq m area and from 6.8 to 272.0 sq m area, respectively were selected for the study.

Prior to application of biolarvicides in tanks and domestic ponds, species-wise larval density per dip of III and IV instar anophelines was measured and recorded. *Bacillus sphaericus* @ 1.0 gm/sq m in six tanks and four domestic ponds and *B. thuringiensis* @ 0.5 gm/sq m in four tanks and two domestic ponds were sprayed through a manual knapsack sprayer fitted with flat fan nozzle. Similarly four tanks and two domestic ponds were taken as control where no biolarvicide application was made. Observations on larval density were made daily in treated and control ponds/tanks for a week and thereafter at weekly intervals upto 6 weeks. Per cent reduction of the larvae over control was calculated as per Mulla's formula[4]. A total 10 anopheline species, viz. *An. culicifacies, An. fluviatilis, An. subpictus, An. annularis, An. nigerrimus, An. vagus, An. barbirostris, An. maculatus, An. gigas* and *An. splendidus* were found breeding in small ponds whereas in cemented tanks breeding of *An. stephensi* was also observed in addition to the above species. Before treatment of biolarvicides larval densities of *An. culicifacies* and *An. fluviatilis* were recorded 0.25 and 0.15 per dip in small ponds and 1.78 and 0.01 per dip in cemented tanks, respectively.

Impact of Spherix

Prior to application of biolarvicides the average anopheline larval density per dip was recorded 31.0 in control and 41.0 in experimental tanks. After application cent per cent reduction of larvae was observed from Day 5 to Day 42 (Fig. 1). In domestic ponds, prior to application of Spherix, density of the larvae was recorded 47.0 whereas in control it was 56.0. After application cent per cent reduction in anopheline larvae was recorded for first three days. From 4 to 28 days, density remained fluctuating and the reduction of larvae varied from 63.3 to 97.5 per cent. Re-application of biolarvicide was made on Day 28 as a result of which density decimated to zero for next two weeks (Fig. 1).

Impact of Bactoculicide

In cemented tanks, initial observation of anopheline density was recorded 30.3 and 28.0 larvae per dip in experimental and control, respectively. After application of biolarvicide per cent reduction in density varied from 70.0 to 96.8 in the initial six days and cent per cent mortality was recorded on Day 7. Though reduction in larval densities were recorded in both experimental and con-

trol tanks on Day 6 and Day 7 but more reduction was observed in experimental tanks. On Day 14, 41.7 per cent reduction of larvae was observed and thus re-application of biolarvicide was made which resulted in cent per cent reduction upto next 4 weeks (Fig. 2).

In domestic ponds prior to application of biolarvicide, anopheline larval density was 18.0 and 9.0, in experimental and control, respectively. Application of Bactoculicide showed 98.4 per cent reduction in larval density on Day 1. Thereafter density remained increasing from Day 2 to Day 14 and the reduction of larvae varied from 9.1 to 96.1 per cent. After re-application of biolarvicide on Day 14, 66.5 to 81.5 per cent reduction in larval population was observed and on Day 42 a very high density was recorded (Fig. 2).

During the study *B. sphaericus* was found effective in cemented tanks from Day 5 to 42 against anophelines. Previous authors have reported impact of Spherix in tanks against anopheline upto 2 weeks[5] and 2 to 4 weeks against *Culex quinquefasciatus* and *Anopheles stephensi* in laboratory and field condition[6]. Bactoculicide impact on anopheline larvae in tanks revealed cent per cent mortality on Day 7 and in domestic ponds larval density was fluctuating up to 42 days. Nearly similar effect of Bactoculicide against *Aedes*, *Culex* and *Anopheles* (ranging from 96 to 100%) for 5 wks was reported earlier[7]. *Ba-*

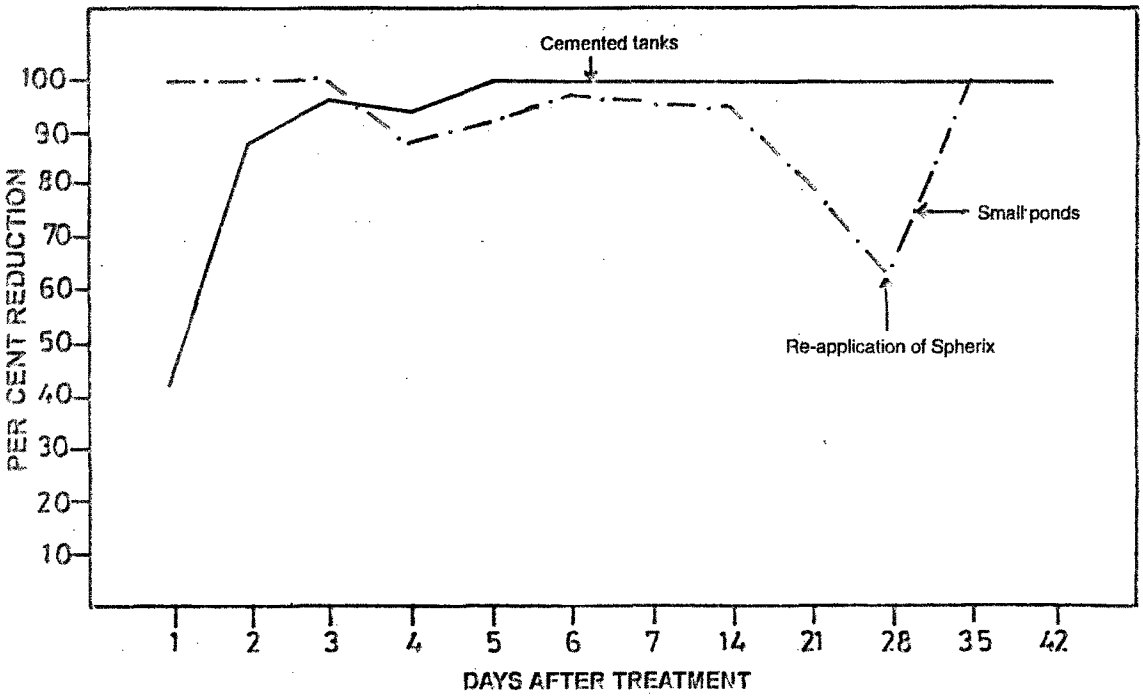

Fig. 1: Larvicidal efficacy of Spherix against anopheline larvae in cemented tanks and small ponds

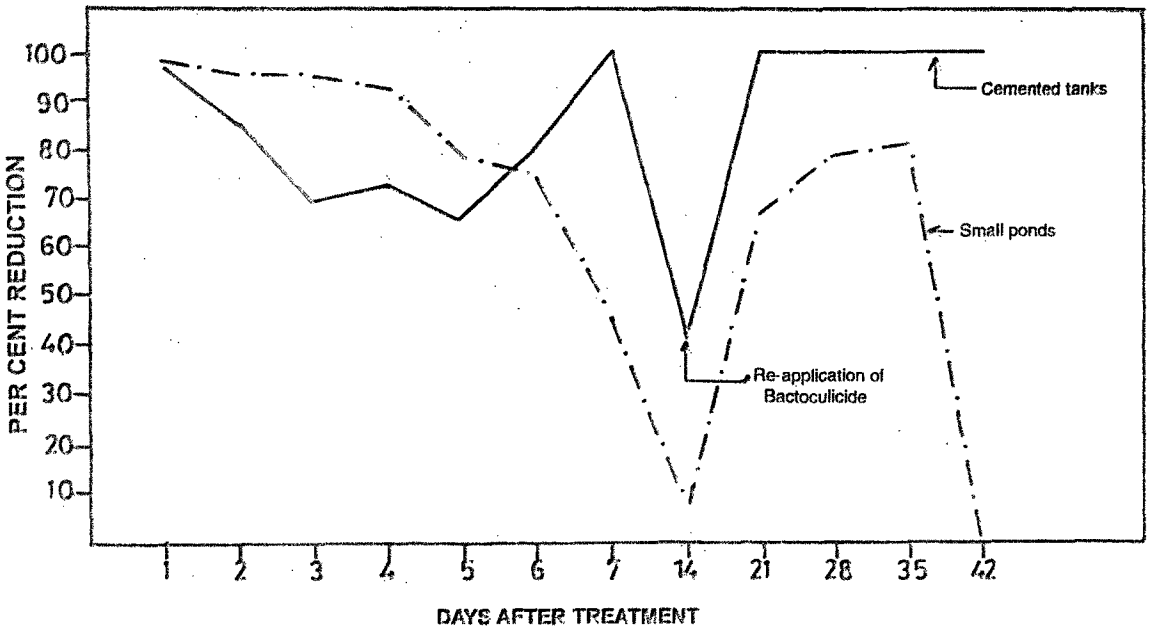

Fig. 2: Larvicidal efficacy of Bactoculicide against anopheline larvae in cemented tanks and small ponds

cillus sphaericus and *B. thuringiensis* being potential biological agents of vector species are reported harmless to non-target organism[1,8].

Our findings thus revealed that *B. sphaericus* is highly effective in cemented tanks up to six weeks but only up to 3 days in domestic ponds against anopheline larvae. *B. thuringiensis* impact on the other hand was observed hundred per cent in tanks against anopheline at Day 7 whereas in domestic ponds its effect on anopheline larvae was very low. This might be due to the fact that small ponds being open are disturbed by the animals and the high vegetation and weeds also obstruct the effectivity of biolarvicide. The impact of Bactoculicide in cemented tanks was less pronounced than that of Spherix against anopheline larvae.

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Fish Fauna of District Raigad, Maharashtra with Particular Reference to Mosquito Larvivorous Species

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Keywords: Fish, Larvivorous, Maharashtra, Mosquitoes

During the post-resurgence era of malaria in India, there has been a revival of interest on the use of biological and environmental measures for control of malaria. Among biological agents fishes like *Gambusia affinis* and *Poecilia reticulata* have been used extensively[1]. During recent years fish fauna surveys have been conducted to explore indigenous fishes that could be used for moquito control[2-4]. Efficacy of certain larvivorous fishes has also been evaluated under field conditions in various habitats[5-9]. Since detailed information on indigenous larvivorous fishes with respect to different ecological zones is still incomplete, there is a need to conduct more exhaustive surveys covering different parts of the country.

District Raigad in Maharashtra is endemic for malaria, therefore, the Malaria Research Centre conducted detailed investigations on malaria during 1995-96 with the objective to study malaria transmission dynamics in the district and suggest appropriate methods of control. Current strategy of malaria control in the district was based on indoor residual spraying with insecticides. In recent years there has been a revival of interest on bio-environmental methods of control incorporating the use of larvivorous fishes[10]. The present study was, therefore, conducted to explore the availability of mosquito larvivorous fishes that could be used locally for msoqutio control in the district.

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Table 1. Fishes of Raigad district and their occurrence in different aquatic habitats

Sl. No.	Species	River	Pond	Irrigation channel	Drain
	Indigenous larvivorous species				
1.	*Amblypharyngodon mola*	+			+
2.	*Aplocheilus lineatus*	A	+	+	+
3.	*Aplocheilus panchax*	+			
4.	*Chela bacaila*	+	+		
5.	*Chela laubuca*	+	+	+	
6.	*Danio aequipinnatus*	P		+	
7.	*Danio rerio*	+			
8.	*Esomus danricus*	+	+		
9.	*Puntius phutunio*	+			
10.	*Puntius sophore*	+			
11.	*Puntius stigma*	+	+	+	
12.	*Puntius ticto*	+			
13.	*Rasbora daniconius*	A	+		
	Other indigenous species				
14.	*Catla catla*		+		
15.	*Channa punctatus*	+	+		
16.	*Cirrhinus mrigala**		+		
17.	*Cirrhinus reba*	+	+		
18.	*Clarias batrachus*		+		
19.	*Gobius* sp	+			
20.	*Heteropneustes fossilis*		+		
21.	*Labeo rohita**		+		
22.	*Lepidocephalichthyes guntea*	+	+		
23.	*Mastocembelus pancalus*	+			
24.	*Mystus cavecius*	+			
25.	*Mystus seenghala*	+			
26.	*Mystus vittatus*	+	+		
27.	*Xenentodon cancila*	+	+		
	Exotic species				
28.	*Cyprinus carpio**		–		
29	*Gambusia affinis*				+
30	*Oreochromis mossambicus**		P		

*Food fish culturable species, P — Plenty; A — Abundant; and + — Present.

In Raigad district on the west-coast, the main river system comprising of Savitri, Kal, Patalganga, Amba and Kundlika rivers provide natural habitats to the local fishes. In addition to rivers and their tributaries, more than 500 tanks/ponds also exist in the district. Random survey of fishes was carried out in March 1996 in rivers, their tributaries, ponds, tanks, canals and drains with the help of a seine net of 10 m x 4 m size. Fishes were also captured with the help of cast net, of about two m diam wherever netting with the help of seine net was not feasible because of the stony river bed. Local fishermen were also contacted to get large carnivorous and edible fishes captured by them.

Table 1 shows the fish fauna and occurrence of various indigenous and exotic larvivorous fishes in different aquatic habitats of Raigad district. In all 30 fish species were found in different aquatic habitats which could be categorized as indigenous larvivorous species (13), other indigenous species (14) and exotic species (3). Most of the fish species were confined to the rivers and ponds. A few species were caught from the irrigation canals and drains. Among the well known indigenous larvivorous fishes *Aplocheilus*, *Danio*, *Rasbora* and *Puntius* species were the most abundant fishes of river ecosystem. *Gambusia affinis* was encountered in the drains of Panvel and Khapoli towns only. However, local authorities reported the presence of large stock of *Gambusia* and guppy fishes in the neighbouring Thane and Ratnagiri districts of Maharashtra. Among the other exotic larvivorous fishes *Oreochromis mossambicus* (Tilapia) was available in towns such as Pen, Panvel and several other village ponds in large number. It was not encountered in other aquatic habitats. In fact, it has been introduced in ponds as a part of edible fish culture in this area.

Among other food fish species found were the Indian major carps, viz. *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala* and an exotic fish *Cyprinus carpio*. All these fishes were being cultured mainly in the municipal and village panchayat ponds on commercial basis.

For the organised biological control programme in the area, mass production of larvivorous fishes is necessary. There are over 540 water tanks in Raigad district with about 1100 ha water spread area. In many of these tanks larvivorous fishes can be cultured along with food fishes. For control purpose it may be necessary to introduce fishes in all permanent and semi-permanent water bodies or habitats. There are more than 14,000 wells including over 4900 irrigation wells in the district as per the record of the Raigad Region Developmental Plan (1988-1989). During the survey 10 (20%) out of 50 wells were checked for mosquito breeding. For control of mosquito breeding in wells it is suggested that locally available larvivorous fishes such as *Aplocheilus* and *Danio* or *Rasbora* species may be systematically introduced. *Danio* fishes have been found valuable in the control of msoquito breeding in rice fields[5]. These fishes could be applied in rice fields of

Mangaon and other areas under paddy cultivation. Larvivorous fishes may also be introduced in the towns and industrial areas in the district.

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