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Short Note

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Field Trials of Combined Use of Two Species of Mermithid Nematodes to Control *Anopheles* and *Culex* Breeding in China

JINZHANG SONG and YUFANG PENG[a]

The field tests of combined use of *Romanomermis yunanensis* 2000-3000 larvae per sq m and *Romanomermis* sp 1000-2000 larvae per sq m in rice fields, ponds and streams in four cities of China, resulted in 60.8-95.5% parasitism in *Culex tritaeniorhynchus*, *Cx. quinquefasciatus*, *Anopheles sinensis* and *An. anthropophagus*. This successful use of two species of *Romanomermis* together not only curb mosquito nuisance it also controls the major vectors of malaria, filariasis and encephalitis B in China.

Keywords: *Anopheles*, *Culex*, Mermithidae, *Romanomermis yunanensis*

INTRODUCTION

In 1984, two species of mermithid nematodes parasitizing mosquitoes were found at the same breeding site in Henan, China[1]. One of them was a parasite of *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* and described as *Romanomermis yunanensis*[2]. The other undescribed mermithid was a parasite of *An. sinensis* and presently designated as *Romanomermis* sp (*R.* sp). A multiple choice host range test indicated that *R. yunanensis* is specific to culicinae; no infection occurred in *An. sinensis*, while *R.* sp selectively infects anophelinae[1]. Since these two mermithids occur simultaneously at one breeding site, the combined use of these two mermithids to control

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culicinae and anophelinae mosquitoes in the same habitat was studied. The results of the field trials are presented here.

MATERIALS AND METHODS

The laboratory stram of *R. yunanensis* and *R.* sp was established from one female and three to five males collected from Henan in 1984 and 1991 respectively and maintained in Song and Peng's laboratory.

With *Cx. quinquefasciatus* as host for *R. yunanensis* and *An. sinensis* for *R.* sp, a mass rearing system of both mermithids was set up to obtain sufficient nematodes for field application[3].

The cultures containing *R. yunanensis/R.* sp eggs were flooded with distilled water for 2 h. Preparasitic larvae (infective larvae) of both mermithids hatched within 8 h were applied simultaneously to the water surface with the help of a compressed air sprayer @ 1000-3000 larvae per sq m depending on the density of the mosquitoes. Breeding sites with target mosquitoes, free of mermithid infections and with no serious water pollution in ecologically diverse regions were selected for infectivity test against *Anopheles* and *Culex*. Mosquito larvae were sampled and dissected one or two days after preparasitic larvae applications. The effectiveness of the applications was evaluated by per cent parasitism. Water temperature was also recorded. In control group, the preparasitic larvae of *R. yunanensis/R.* sp were released alone, not in combination.

RESULTS

The field trials of synergic use of two *Romanomermis* were conducted in Sichuan, Guizhou and Guangxi province of China from 1992-94. At the rate of 2000-3000 larvae per sq m of *R. yunanensis* and 1000-2000 larvae per sq m of *Romanomermis* sp were released simultaneously in rice fields, ponds and streams; resulting in 60.8-76.9% and 68.6-95.5% parasitism in *Culex* and *Anopheles* respectively (Table 1). In control group, 66.1% parasitism in *Cx. tritaeniorhynchus* and none in *An. sinensis* was noticed, when 3000 larvae per sq m of *R. yunanensis* was used alone. Whereas, 77.8% parasitism in *An. sinensis* was observed when 2000 larvae per sq m of *Romanomermis* sp was also released. Therefore, the mixed infection of these two mermithids has not been observed in their natural host *An. sinensis* and *Cx. tritaeniorhynchus.*

DISCUSSION

All infected mosquito larvae die after the post-parasitic mermithid emerged thus, levels of parasitism were indicative of the number of mosquito killed by the nematode application. The field trials of combined use of *Romanomermis yunanensis* 2000-3000 larvae per sq m and *Romanomermis* sp 1000-2000 larvae per sq m in various ecological re-

Table 1. Field test of combined use of two *Romanomermis* for control of *Anopheles* and *Culex*

Province/Habitat	Temperature (°C)	Area (sq m)	No. of preparasitic larvae per sq m		*An. sinensis*		*An. anthropophagus*	
			Romano-mermis sp	*Romano-mermis yunanensis*	No. mosquito examined	% parasitism	No. mosquito examined	% parasitism
Guiyang, Guizhou Stream	25-31	16	1800	3000	67	95.5	–	–
Guilin, Guangxi Pool	28-37	12	2000	2500	55	85.5	–	–
Chengdu, Sichuan Pond	25-32	60	1000	2000	–	–	116	68.8
Emeishan, Sichuan Paddy field	24-30	41	2000	3000	116	75.9	–	–
Paddy field	24-30	15	2000	3000	80	70.0	–	–
Paddy field	24-30	10	2000	3000	70	68.6	–	–
Pool	24-28	1.2	2000	3000	108	87.0	–	–
Marsh	24-28	11	2000	3000	82	82.9	–	–
Control , Paddy field	24-30	10	2000	0	108	77.8	–	–
Control ., Paddy field	24-30	10	0	3000	112	0.0	–	–

contd...

Table 1 (*contd.*)

Province/Habitat	Temperature (°C)	Area (sq m)	*Cx. tritaeniorhynchus*		*Cx. quinquefasciatus*	
			No. mosquito examined	% parasitism	No. mosquito examined	% parasitism
Guiyang, Guizhou						
Stream	25-31	16	61	73.8	66	63.6
Guilin, Guangxi						
Pool	28-37	12	54	68.5	-	-
Chengdu, Sichuan						
Pond	25-32	60	-	-	63	60.8
Emeishan, Sichuan						
Paddy field	24-30	41	80	65.0	-	-
Paddy field	24-30	15	64	71.9	-	-
Paddy fied	24-30	10	68	64.7	-	-
Pool	24-28	1.2	104	76.9		
Marsh	24-28	11	104	75.0	-	-
Control , Paddy field	24-30	10	73	0.0	-	-
Control , Paddy field	24-30	10	118	66.1	-	-

gions in China, resulted in 60.8-95.5% parasitism in *Culex* and *Anopheles* (Table 1). This indicates that two mermithids cooperated to kill *Anopheles* and *Culex* simultaneously in the same breeding site.

Mosquitoes such as *An. sinensis*, *An. anthropophagus*, *An. dirus*, *An. minimus*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus* etc., are the major vector for malaria, filariasis, dengue and encephalitis B in China. Rice fields, ponds and streams are the major breeding sites for these mosquitoes. At present, no single species of mermithid that can control both *Anopheles* and *Culex* simultaneously are known. Although *Romanomermis culicivorax* has a broad host range and has been demonstrated as a good biological control agent[4-6] it doesn't parasitize *An. sinensis* and is not highly effective against culicinae under field conditions[7,8]. Theoretically, it would be optimal to combine these traits in a single species, but at present our understanding of nematode hybridization[9] or genetic recombination is insufficient to produce such a hybrid. Therefore, combined use of two species of mermithid for the control of various species of mosquito simultaneously might be a practical method today.

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Clinical Trials of a New Immunochromatographic Test for Diagnosis of *Plasmodium falciparum* Malaria in Goa

ASHWANI KUMAR, V.P. SHARMA[1], D. THAVASELVAM and P.K. SUMODAN

Plasmodium falciparum histidine rich protein-2 (PfHRP-2) based immunochromatographic test kit (ICT Malaria Pf) for the rapid diagnosis of *P. falciparum* malaria was evaluated at the clinic of Malaria Research Centre (Field Station), Goa. Of the 98 febrile patients screened, 22 were ICT positive for *P. falciparum*. Simultaneous microscopic examination of the blood smears of these ICT positive patients showed that 20 were positive for *P. falciparum* alone, whereas one had mix infection of both *P. vivax* and *P. falciparum* suggesting 100% sensitivity. Only one slide negative patient who had taken 600 mg chloroquine the previous day was positive in the ICT. Out of the remaining 76 blood smears, 41 showed *P. vivax* infection and none cross-reacted with *P. falciparum* HRP-2 antigen and were ICT negative except one mix infection case in which *P. vivax* and *P. falciparum* infections occurred concomitantly suggesting species specificity of 98.7%. The positive predictive value, negative predictive value and efficacy of the ICT were 95.4, 100 and 98.9% respectively. The band intensity of the ICT positive cases significantly correlated with *P. falciparum* parasitaemia ($p<0.01$). The usefulness and the disadvantages of this diagnostic kit have been discussed in context of prevailing malaria situation in the country.

Keywords: Clinical diagnosis, Goa, Immunochromatographic test, *P. falciparum*

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INTRODUCTION

The National Malaria Eradication Programme of India reports 2-3 million cases of malaria annually[1]. The proportion of the deadly *P. falciparum* species has been gradually rising in the country over the years and presently 39% of all the cases reported belong to this species. In the vast areas of the country that are inhabited by the ethnic tribes, meso- to holo-endemic malaria conditions exist and *P. falciparum* species accounts for approximately 52% of the total malaria cases[2]. These areas also have accessibility and logistical problems and delays in the radical treatment of malaria due to delayed diagnosis are common.

The diagnosis of malaria is primarily based on microscopic examination. It is time consuming and requires preparation of blood smears, their transportation to the laboratory, staining and examination by a skilled technician. The whole procedure, at times might take many days and back log of unexamined slides is not a uncommon feature. The other recently evolved diagnostic methods are antigen-capture enzyme linked immunosorbent assay (ELISA)[3] and dipstick test (PARASIGHT®-F)[4]. Although the latter method is fairly quick yet multiple manipulations required in the test may be a constraint in the field. With the new immunochromatographic test which is a simple and rapid PfHRP-2 based field diagnostic method for *P. falciparum* detection, these constraints could be overcome. We therefore evaluated ICT Malaria Pf® test kit in Goa to find out its sensitivity, specificity and efficacy in the detection of *P. falciparum* malaria. The results are presented in this paper.

MATERIALS AND METHODS

The ICT Malaria Pf kit is based on *P. falciparum* histidine rich protein (PfHRP-2) antigen and sheep polyconal antibody reaction[5]. The *P. falciparum* parasites synthesize water soluble histidine rich protein which is released from *P. falciparum* infected erythrocytes.

The immunochromatographic Pf test kits were supplied through the courtesy of M/s. ICT Diagnostics, 3/14 Roseberry, St. Balgowlah, NSW 2093, Australia. The kit consists of a labelled cardboard device with opposable flaps (Fig. 1). The right flap of the device has 4 cm long test strip with a fine fibrous pad below a purple band containing gold labelled polyclonal antibodies. About an inch above this band is a limit line that is indicated by a broken line. On the right margin of this flap is a 3/4 inch broad adhesive lining to secure the flaps firmly together after the test has been performed. On the left flap is a large absorbent fibrous pad of 2.5 x 1.7 cm area and a small upper blood clearing pad of one sq cm area. In between these two pads is a viewing window to read the test results. Each test kit is contained in foil pouch with a desiccant.

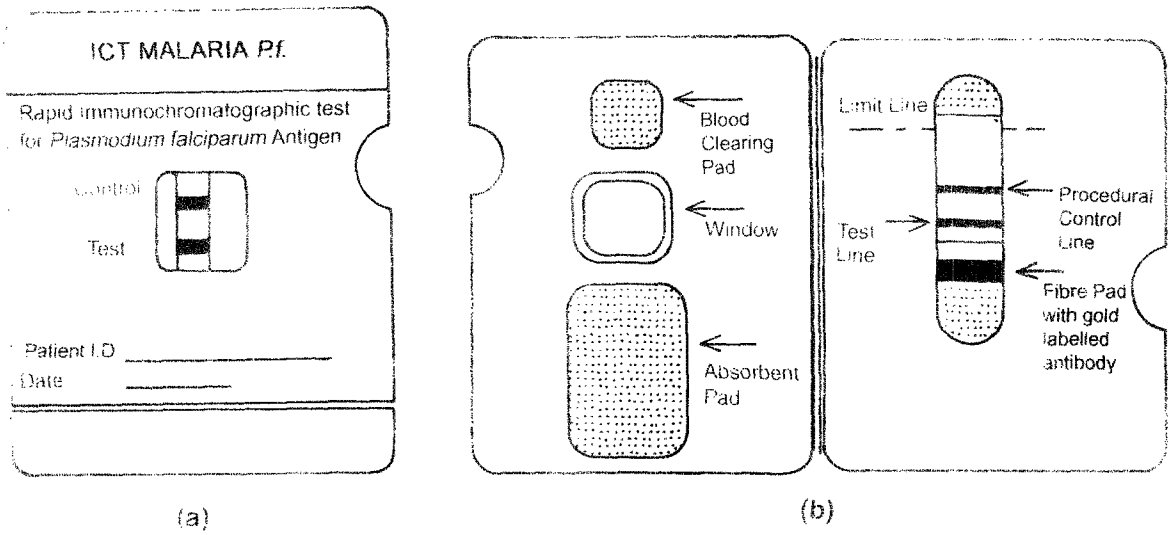

Fig. 1: ICT kit (a) closed, (b) open

The kits were stored at 4°C prior to use. The test was performed on 98 febrile patients in the clinic of the Malaria Research Centre, Goa from May 7-27, 1996. The index finger of the patient was pricked with disposable lancet and blood was taken up to 3/4 (ca. 10 µl) of EDTA coated capillary tube provided with the kit. The blood was released by gently pressing the tip of capillary across the purple band containing gold labelled polyclonal antibodies. Two drops of the buffer (provided in the kit box as reagent A) were added to the pad just below this band. The blood was allowed to migrate up till limit line after which four drops of buffer were added on the clearing pad. The adhesive band was then exposed and both the flaps were secured firmly against each other. The blood was allowed to clear up for reading the results through the window. The procedural control line appeared in all except 2 kits and in these cases the test was repeated. The test lines were graded according to the band intensity: +1 = Barely discernible; +2 = Readable; +3 = Easily readable; and +4 = Intense.

Microscopic examination: Both thick and thin blood smears of all 98 test patients were made simultaneously while performing immunochromatographic test. They were stained with 10% Giemsa and examined under a compound microscope at 1000x magnification. The *P. falciparum* density was calculated using the following formula.

$$\text{Parasite/ µl blood} = \frac{\text{No. of parasites counted}}{\text{No. of WBC counted}} \times \text{Av. WBC count/µl}$$

The average WBC count of population was obtained by examining 100 slides of persons representing various age groups. The results were further interpreted with the help of the following formulae:

$$\text{Sensitivity} = \frac{A}{A+C} \times 100$$

$$\text{Specificity} = \frac{D}{D+B} \times 100$$

$$\text{Positive predictive value} = \frac{A}{A+B} \times 100$$

$$\text{Negative predictive value} = \frac{D}{D+C} \times 100$$

$$\text{Efficacy} = \frac{A+D}{A+B+C+D} \times 100$$

Where, A = No. positive both microscopically and for ICT; B = No. negative for ICT but microscopically positive; C = No. negative microscopically but positive for ICT; D = No. negative both microscopically and for ICT.

To find out relationship between *P. falciparum* parasitaemia and test band intensity, correlation coefficient was worked out and its significance was tested.

RESULTS

Table 1 shows the results of the immunochromatographic test obtained in 98 febrile patients. Of these 22 were ICT positive for *P. falciparum*. The microscopic examination of these patients showed that 62 slides were positive for malaria parasites; 20 for *P. falciparum*, 41 for *P. vivax* and one for mixed infection of *P. vivax* and *P. falciparum*. All the 21 patients who were slide positive for *P. falciparum* were also positive in the ICT and no cross-reactivity with *P. vivax* antigens was observed. Similarly, all the slide negative patients were also negative for ICT except one *P. falciparum* patient who took 600 mg chloroquine the previous day of the test and was ICT positive but microscopically negative. The test efficacy, sensitivity and specificity were high being 98.9, 100 and 98.7% respectively. The positive and negative predictive values of the test were 95.4 and 100% respectively.

Of the 22 ICT *P. falciparum* patients, in 5 the test line was barely discernible and asexual parasitaemia ranged from 352 to 2797/µl of blood ($\bar{x}$ = 944.8). In another 5 cases the test lines were readable and parasitaemia ranged from 310 to 5206/µl ($\bar{x}$ = 2424). In 10 cases the lines were easily readable and parasitaemia ranged from 452 to 3732/µl ($\bar{x}$ = 2150.7) except in one slide negative *P. falciparum* patient who received chemotherapy prior to the test. In the

Table 1. Results of ICT for *P. falciparum* diagnosis *vs.* microscopic examination

ICT test			Microscopic diagnosis			
Total tested	(+)ve *Pf*	(-)ve	(+)ve *Pf*	(+)ve *Pv*	(+)ve *Pf* and *Pv*	(-)ve malaria
98	22	76	20	41	1	36

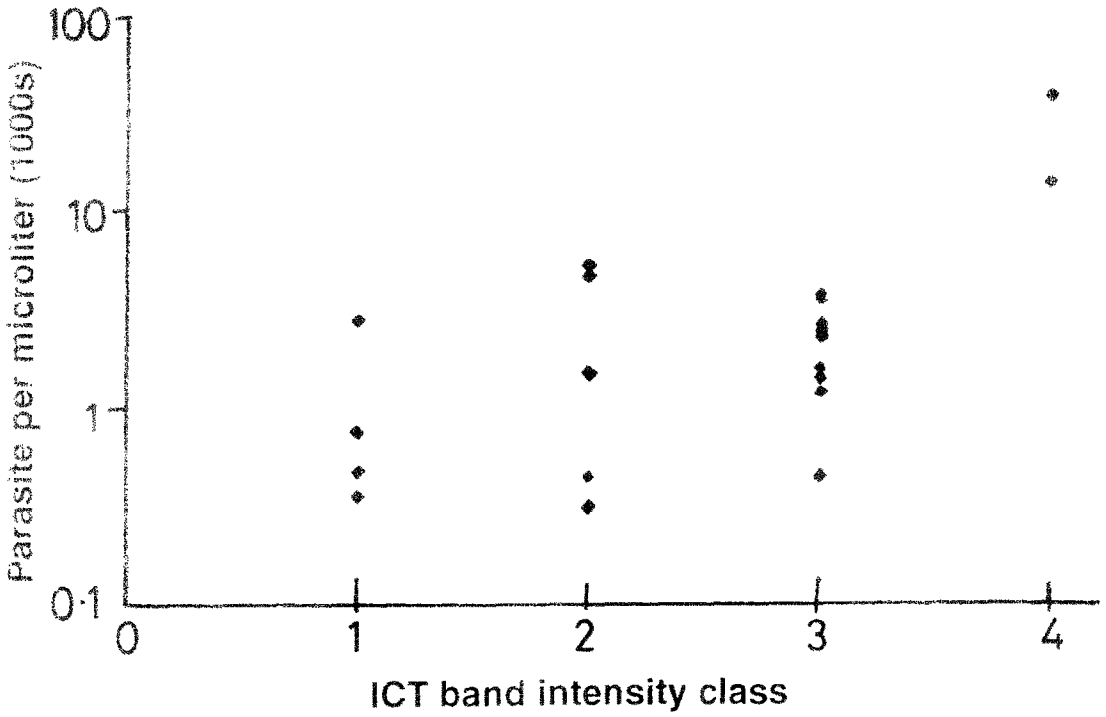

Fig. 2: Reationship of ICT band intensity and *P. falciparum* parasitaemia

remaining two cases, the test lines were intense and parasitaemia in these cases was 14,004 and 38,900/µl of blood respectively. The lowest parasitaemia in this study at which the test showed positivity was 352 parasites/µl of blood and the highest was 38,900/µl.

When the parasite density was correlated with the intensity of test lines in the cases which were both slide and ICT positive, the correlation was positive ($r = +0.52$) and significant at $p<0.01$ level which means that band intensity is dependent upon the level of PfHRP-2 circulating antigen produced by the parasites (Fig. 2).

DISCUSSION

The ICT kit for the diagnosis of *P. falciparum* malaria is reliable due to its high sensitivity, specificity, predictive values and efficacy observed in the present study. The test kit can significantly improve detection of *P. falciparum* in all epidemiological situations particularly in areas with stable hyper- and holo-endemic malaria where asymptomatic cases are also encountered due to enhanced immunity. Such cases usually go undetected and play significant role in maintaining transmission of *P. falciparum*. The test is fast and the results can be read even

before a thick blood film simultaneously prepared dries up for staining and examination.

The basic disadvantage is that the test is positive even in the treated *P. falciparum* aparasitaemic cases due to circulating PfHRP-2 antigens[5]. In the present study also an aparasitaemic patient treated on the previous day with chloroquine was strongly positive for the ICT. Further studies are needed to know by what day post-treatment *P. falciparum* patients become negative for PfHRP-2. Moreover, in case of mixed infection of *P. vivax* and *P. falciparum*, the primaquine regimen meant for *P. falciparum* administered on the basis of ICT would not be adequate to prevent relapse of *P. vivax*. The storage of kits at 2-8°C is another disadvantage in the field conditions.

In the present form, the kit cannot completely substitute microscopic diagnosis of malaria as *P. vivax* detection would still require microscopical examination of blood smear. Therefore, under proper storage facilities, a similar kit meant for detection of both *P. vivax* and *P. falciparum* represented by two separate test lines would be far more advantageous over the present kit as it would be useful in *P. vivax* predominant areas as well.

Nevertheless, the kit is useful in remote and poorly accessible *P. falciparum* predominant areas. It will also be of great diagnostic value in the early detection of complicated *P. falciparum* cases admitted in the hospitals. Due to its high sensitivity, the immunochromatographic test could also be advantageous in the cases with extremely low and sub-microscopic parasitaemia which is though a rare possibility except in immune subjects harbouring *P. falciparum* infection. The test is simple, easily readable, reliable and can be performed readily in the laboratory and field. The treatment of the detected cases can be instituted almost immediately. This will not only prevent morbidity and mortality but also have a good impact on *P. falciparum* transmission especially in the areas of its predominance.

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Studies on *Plasmodium vivax* Relapse Pattern in Kheda District, Gujarat

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Relapse pattern in *P. vivax* malaria was studied in five villages of Nadiad taluka, Kheda district, Gujarat. *P. vivax* cases treated with 600 mg chloroquine and in combination with 50 mg pyrimethamine (adult dose) yielded 28.31 and 27.73% relapse rate respectively. While relapse rate of 5.78% was observed with five day course of 75 mg primaquine (15 mg/day) administered as radical treatment. Relapse rate in 5-10 yr of age group was comparatively more than other age groups. There was no noticeable difference in relapse rates among male and female. The short-term relapse with a lag period of 2-3 months was significantly higher in this area. The longest relapse with an interval of 17 months was found in one case treated with chloroquine along with pyrimethamine. However, primaquine regimen prevented consecutive relapses. Primaquine has been found adequate to prevent relapse in more than 90% vivax cases, while efficacy of chloroquine-pyrimethamine and chloroquine alone was almost comparable. A high proportion of relapse may be minimized, if 5-days radical treatment is given at appropriate time.

Keywords: Hypnozoite, *P. vivax*, Pyrimethamine, Relapse, Sporozoite

INTRODUCTION

Plasmodium vivax, malaria though not an intrinsically life threatening disease, is important because of the morbidity and debility it produces as a result of relapse and frequent infections. The difference in strains and their biologi-

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such characteristics may be the probable cause of relapse[1]. In recent years chemotherapy of malaria has assumed a major role in malaria control programme to prevent morbidity and mortality. Primaquine is the only effective drug used against *P. vivax* as anti-relapse since beginning. It was presumed that prevention of relapse would automatically cutdown the carrier rate and would thus minimize the possibility of further transmission. In early sixties the efficacy of primaquine has been always a subject of major concern. However, relapse rate with primaquine was found to be 5% in 1953[2]. Subsequently, other authors from various parts of India have studied the relapse rate and efficacy of primaquine[3-6]. Relapse rate of *P. vivax* was observed to be 2.6% with 5-day primaquine regimen in District Kheda, where a project on bioenvironmental control of malaria was in operation from 1984-89[7]. The present study was carried out to find the relapse pattern, frequency and efficacy of radical treatment in *P. vivax* malaria and the results are presented in this paper.

Study area

Study was carried out in five villages of Nadiad taluka of District Kheda, Gujarat. Population of five villages is 22,820 with 31.12% scattered houses in hamlet/fields. It has 23.99 sq km canal irrigated area which is 75% of the total land under cultivation. Paddy is cultivated twice a year during February-May and July-October. Other major crops are millet (*bajra*), tobacco and wheat. The rainfall in this area is uncertain, however average rainfall is 769.5 mm and mean temperature ranges between 20.41-32.76°C in January and May respectively.

MATERIALS AND METHODS

For the purpose of investigation, the villages were grouped into three areas namely, Davda and Dantali in area I, Bamroli and Pij in area II and Tundel in area III. Weekly surveillance was organized to detect malaria cases by a trained resident surveillance worker of each village. Blood smears of all fever cases and those having history of fever in last eight days were examined and presumptive treatment (600 mg chloroquine as adult dose) was given to each patient. Blood smears were stained with JSB stain and examined. All *P. falciparum* malaria positive cases were given radical treatment as per NMEP drug schedule. Whereas, *P. vivax* cases in three areas were administered drugs as 600 mg chloroquine alone in area I; 600 mg chloroquine and 75 mg primaquine (15 mg/day) in area II; 600 mg chloroquine along with 50 mg pyrimethamine in area III. It is believed that pyrimethamine has an effect on primary exo-erythrocytic stage and combination of chloroquine and pyrimethamine was also used as preventive treatment[8]. Hence, this combination was used in present study to compare its efficacy against chloroquine alone. All *P. vivax* cases were followed up weekly. Blood smear of each vivax

case was prepared once a month or in case if there was a complaint of fever during surveillance. Periodicity was also observed clinically in relapse cases by which they could be separated from fresh infections.

RESULTS AND DISCUSSION

The malaria situation in the study area remained somewhat stationary during the study period, with the slide positivity rate (SPR) ranging between 9.9 and 10.9%. The proportion of *P. vivax* was almost 58% of the total malaria cases (Table 1). Slide positivity rate was lowest in area II, whereas in area III. *P. vivax* infections were maximum throughout the study period. Sex-wise distribution of vivax malaria incidence yielded no significant difference in male (53.84%) and female (46.15%). The population above 14 yr of age suffered maximum with *P. vivax* infection (58.52%) followed by 5-10 yr of age (23.52%). However, *P. vivax* prevalence was significantly higher in this group than other age groups of children, supporting previous observation[9]. A total of 226 cases from area I, 173 from area II, and 136 cases from area III were followed up for relapse, where chloroquine, chloroquine with primaquine, and chloroquine along with pyrimethamine were administered respectively. 28.3 per cent cases relapsed when treated with chloroquine alone, 27.73% with chloroquine + pyrimethamine, and 5.78% with chloroquine + primaquine with mean relapse

Table 1. Malaria incidence in study areas

Study area	Population	Year	BSE	(+)ve	Pv	SPR	SvR	API
I	7,347	1992	3081	334	111	10.84	3.60	45.4
		1993	1395	170	117	12.18	8.38	23.13
II	12,098	1992	2895	240	166	8.29	5.73	13.83
		1993	1551	139	128	8.96	7.93	11.48
III	3,375	1992	2047	222	113	10.84	5.52	65.77
		1993	650	83	68	12.76	10.46	24.59
Total	22,820	1992	8023	796	390	9.92	4.86	34.88
		1993	3596	392	308	10.90	8.56	17.17

Table 2. Relapse rate in *P. vivax* cases treated with different drug regimens

Months	Primary infection			Relapse			Relapse rate		
	CQ	CQ+ PQ	CQ+ PYR	CQ	CQ+ PQ	CQ+ PYR	CQ	CQ+ PQ	CQ+ PYR
Jan	1	0	2	1	0	0	100.00	0.00	0.00
Feb	4	0	2	1	0	0	25.00	0.00	0.00
Mar	6	1	4	0	1	2	0.00	100.00	50.00
Apr	15	10	10	9	1	4	60.00	10.00	40.00
May	17	15	12	6	1	5	35.29	6.67	41.67
Jun	13	16	13	4	0	3	30.77	0.00	23.08
Jul	14	13	12	2	2	4	14.29	15.38	33.33
Aug	27	18	16	10	0	3	37.04	0.00	18.75
Sep	95	79	48	22	4	15	23.16	5.06	31.25
Oct	26	15	12	6	1	2	23.08	6.67	16.67
Nov	8	5	5	3	0	0	37.50	0.00	0.00
Dec	0	1	0	0	0	0	0.00	0.00	0.00
Total	226	173	136	64	10	38	28.31	5.78	27.73
Mean				5.33 (5.96)	0.83 (1.14)	3.17 (3.95)			

CQ — Chloroquine; PQ — Primaquine; PYR — Pyrimethamine; Figures in parentheses represent the SD values.

5.33±5.96, 3.17±3.95, and 0.83±1.14 respectively (Table 2). The observation with chloroquine along with primaquine is in conformity with those reported earlier[2,3,6]. However, low level of relapse has been reported earlier form the present study area[7]. The relapse rate of vivax malaria in Delhi with chloroquine regimen ranged from 23-44% depending on duration of follow-up[10]. There was no significant difference in relapse rate between chloroquine and chloroquine with pyrimethamine regimens, reflecting similar potential of chloroquine-pyrimethamine on *P. vivax*. Seasonal prevalence of relapse was more variable, but showed an overall trend to decrease in post-monsoon season.

It is inferred from Table 3 that maximum relapses occur in younger age with highest rate of 32% in 5-10 yr of age group. Relapse rate declined with the increase in age which is evident from the analysis of data. Thus relapse rate was observed to be 17.6% in 15-30 yr of age group which found to be 13.2% among adult above 30 years of age. Such trend of relapse might be

associated with increase in anti-disease immunity with age in adults.

In general, maximum relapses (42.8%) occurred within three months lag period, while minimum relapse (8%) was observed after 12 months (Table 4). The data revealed that patients treated with chloroquine and chloroquine along with pyrimethamine had maximum relapses within 2-3 months, whereas those treated with primaquine had more relapses after 4-6 months. This comparison among lag periods of relapses after treatment with different regimens, was found statistically significant (χ^2 = 20.32, $p < 0.01$). Results of the present investigation indicate more than 90% relapses in a year and are comparable with reports from Nepal[11] and Hardwar[3]. However, the longest lag period of 17 months was observed in one case treated with chloroquine-pyrimethamine. Similarly, relapses with different regimens of primaquine have been reported to occur within six months in Thailand[12]. This suggest that the use of primaquine do not alter

Table 3. Age and sex-wise distribution of relapse rate

Sex	Age group (yr)					
	2-4	5-10	11-14	15-30	>30	Total
Male	15.8	34.3	30.0	14.4	13.7	20.5
Female	26.0	29.6	17.4	22.2	12.7	21.4
Total	21.4	32.0	24.5	17.6	13.2	20.9

Table 4. Duration of lag period in *P. vivax* relapse

Area	No. of cases	Drug administered	Lag period (days)					No. of relapse cases
			28-90	91-180	181-270	271-365	>365	
I	226	Chloroquine	28 (43.7)	5 (7.8)	15 (23.4)	14 (21.9)	2 (3.1)	64
II	173	Chloroquine + Primaquine	2 (20.0)	4 (40.0)	-	2 (20.0)	2 (20.0)	10
III	136	Chloroquine + Pyrimethamine	18 (47.3)	7 (18.4)	6 (15.8)	2 (5.3)	5 (13.1)	38
Total	535		48 (42.8)	16 (14.3)	21 (18.7)	18 (16.1)	9 (8.0)	112

χ^2-20.32, df-8, $p<0.01$; Figures in parentheses are in per cent.

the pattern of relapse characteristics of *P. vivax*.

Three consecutive relapses were found in two patients one each with chloroquine and chloroquine along with pyrimethamine treatment. Two consecutive relapses were observed in 8 (12.72%) and 9 (28.51%) cases with similar regimen. In case of primaquine treated persons no consecutive relapse was observed. This reflects satisfactory efficacy of primaquine against hypnozoites of *P. vivax*.

The investigation revealed that present radical treatment of *P. vivax* malaria in this area is adequate for more than 90% cases and may be continued. The frequency of relapse may be minimized with early radical treatment. While those not responding to present primaquine regimens may be under the influence of other factors such as duration of incubation period, period of latency, immunity status of the patient, quantum of sporozoites and distinct population of sporozoites[10,13,14]. However, higher dose of primaquine may be tried keeping in view its propensity to induce haemolysis in patient with G-6-PD deficiency. Two distinct patterns of relapses in this area confirm the existence of *P. vivax* strains with different lag period of relapse. In general all relapses within three months may be of short-term incubation period. The consecutive relapse after primaquine therapy in *P. vivax* malaria during present investigation needs further confirmation in this area.

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Biting Rhythms of Some Anophelines in Central Gujarat

R.M. BHATT and V.K. KOHLI[a]

A total of 41,552 anophelines comprising 16 species were collected during 70 all-night bovine-bait collection carried out in six villages of Kheda district, Gujarat. *Anopheles subpictus, An. varuna, An. culicifacies, An. stephensi* and *An. vagus* had unimodal biting rhythms. Most feeding occurred during the early night with occasional increase during pre-dawn/dawn hours. *An. pallidus* exhibited bimodal biting rhythm with two well-defined peaks. *An. turkhudi, An. tessellatus, An. fluviatilis, An. aconitus, An. annularis; An. barbirostris* and *An. nigerrimus* had multimodal biting rhythms or were arhythmic. *An. culicifacies, An. varuna, An. aconitus* and *An. tessellatus* exhibited a marked seasonal shift in feeding activities with most biting occurring at dusk in colder months and late at night during warmer months.

Keywords: Anophelines, Biting rhythm, Gujarat

INTRODUCTION

Information on the biting rhythms of mosquitoes, especially vector species is essential for determining the period of disease transmission and maximum biting activity to schedule sampling efforts for various studies. Although, in past many studies have been conducted on ecology and bionomics of the anophelines in India but few described biting rhythms in detail.

Viswanathan *et al.*[1] made all-night collections on bovine baits in specially constructed huts and studied the biting activity of *An. culicifacies* in a rural area near Pune. *Reuben*[2] presented

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observation on 18 species common in Madras (now Tamil Nadu) state. Kulkarni[3] studied the feeding behaviour of anopheline mosquitoes in Bastar district, Madhya Pradesh. In Pakistan, Reisen and Aslamkhan[4] studied the biting rhythms of some mosquitoes using bovine-baits and showed the seasonal shift in the feeding times of different species. The present study of all-night bovine-bait collections was undertaken to understand the biting rhythms of some anophelines in Kheda district, Gujarat.

MATERIALS AND METHODS

All night bovine-bait collections were made in six villages belonging to three different physiographic areas (ecological zones) namely, canal irrigated (Davda, Pansora), non-canal irrigated (Sapla, Vanthvadi) and riverine (Sanali, Raniya). The areas have been described in detail earlier[5]. Beginning from January 1989, collections were made once a month for one year in each village except in Vanthvadi village, where collection could not be made during July and August. Outdoor bednet trap collections were made in the last quarter of each hour from 1800-0600 hrs using bullock as a bait[5]. Some species of anophelines were caught in significantly large numbers on the bullock than on buffalo[2] and linear relationship has been suggested between host weight and range of attraction of mosquitoes[6]. Thus to avoid any discrepancy in collection of mosquitoes due to differential attraction based on type and size of the bait, only white coloured adult bullock was used as bait in all collections. Collections were made preferably at a fixed site and in the first fortnight of every month in each village.

Fully-fed females resting on the inner surface of the net were collected, transferred to mosquito cages after each hours collection and only anophelines were identified[7]. Hourly collections of each village were pooled for each month and biting rates were computed as logarithmic transforms known as William's mean (Mw), expressed in percentage[8]. Biting rhythms of various species have been presented for those months only when the total number collected exceeded 10 per bait per night[4].

RESULTS AND DISCUSSION

A total of 41,552 anophelines comprising 16 species were collected from 70 all-night bovine-bait collections. *An. subpictus* was most abundant (61.7%), followed by *An. aconitus* (8.9%), *An. nigerrimus* (6.4%), *An. culicifacies* (5.5%) and *An. annularis* (5.5%).

An. aconitus was most abundant during June and July (Fig. 1). Biting during colder months started as early as 1800 hrs. Maximum feeding took place during the second half of the night almost throughout the year. There was a slight seasonal shift in peak biting activity from third quarter of the night during June-July to the last quarter during August to October. Biting curves for the months when feeding com-

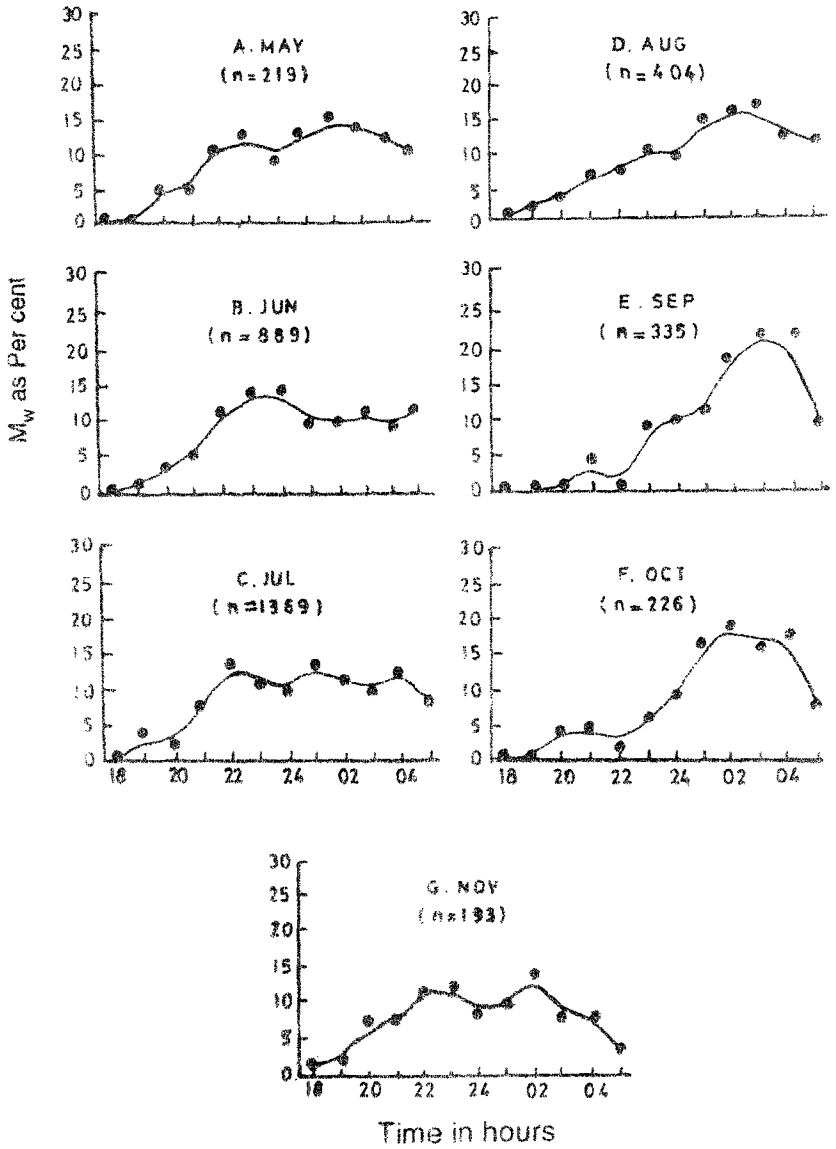

Fig. 1: Biting rhythms of *An. aconitus* during May-November

menced during dusk hours conforms to the observations made in Myanmar[9] and Madhya Pradesh[3].

An. annularis was collected throughout the year and it was most abundant during March, August and September. During March-April peak biting occurred in the first and second quarters of the night. From June to September more specimens were collected after midnight and biting was arhythmic. It

was active throughout the night during all the months. The biting rhythms for the warmer months are in agreement with reports from Orissa[10] but differ from the observations reported from Pakistan[4], where this species is crepuscular and most of the biting occurred between dusk and 2030 hrs. No marked shift in its biting time was observed.

An. barbirostris was collected throughout the year with very high catches from July to October (Fig. 2). During March-April maximum biting occurred in the first quarter which shifted to second in June. Small increase in the activity was observed at dawn. *An. barbirostris* was found to be active throughout the night except during November. Similar observations have been made in Myanmar[9].

An. culicifacies was collected throughout the year and was most abundant during February-April (Fig. 3). It ex-

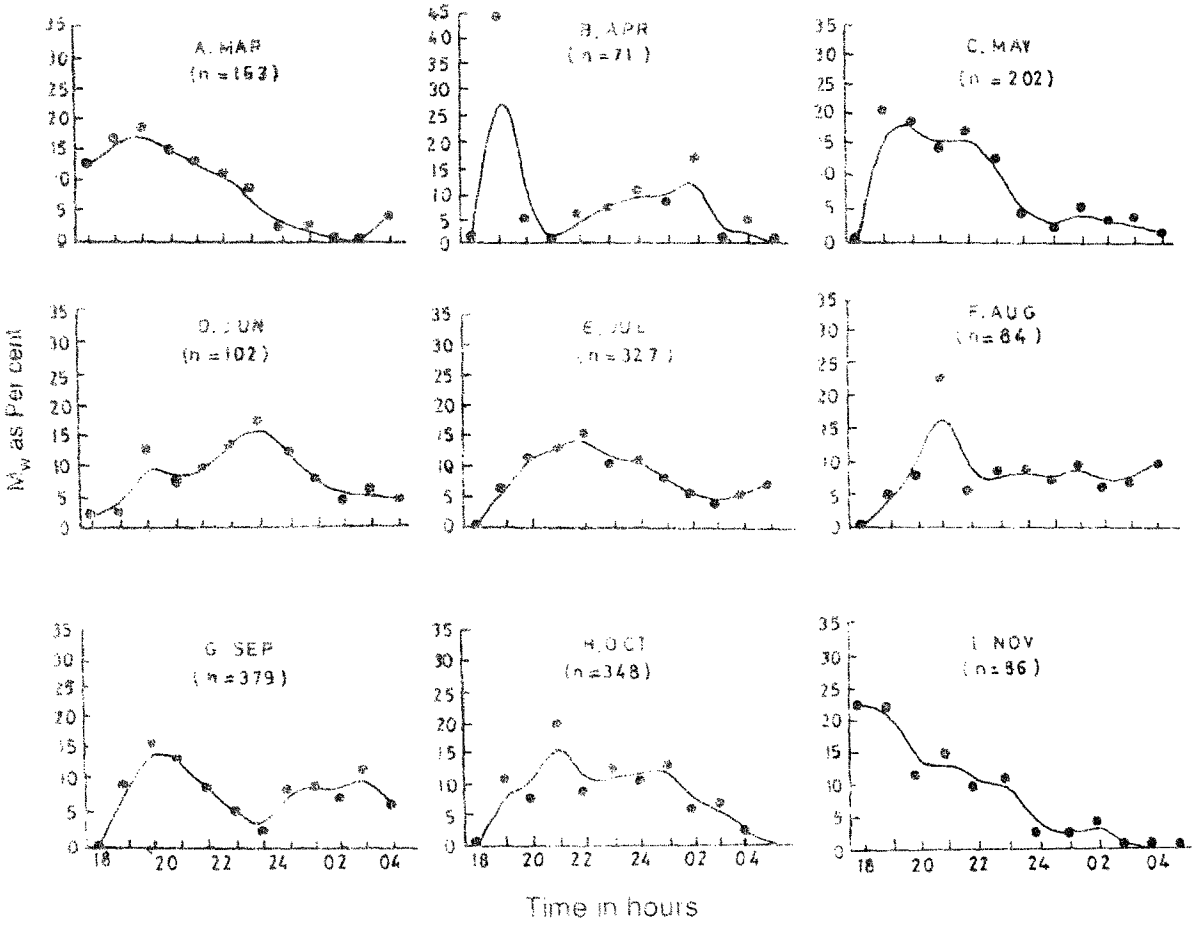

Fig. 2: Biting rhythms of *An. barbirostris* during March-November

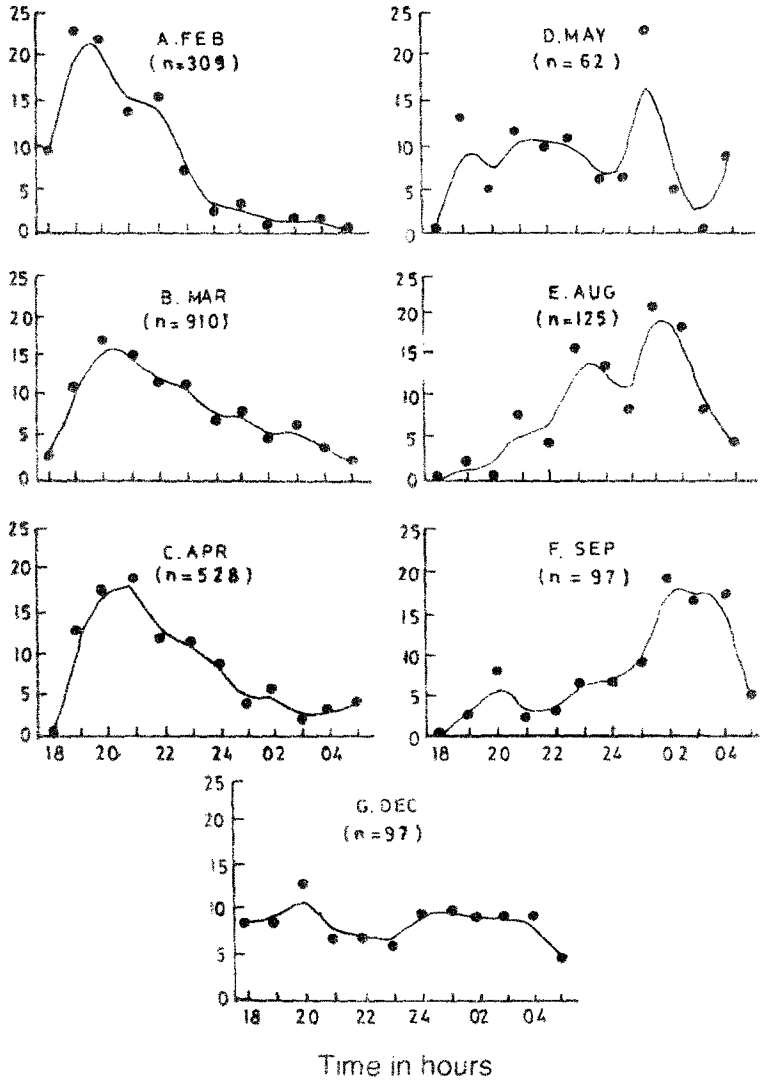

Fig. 3: Biting rhythms of *An. culicifacies* during February, May, August-September and December

exhibited marked seasonal changes in its feeding time. During February-April most biting occurred in the first and second quarters of the night with peak activity shifting by one hour from 1900 hrs in February to 2100 hrs in April. During warmer nights (May to September) feeding was late-night and maximum biting took place in third quarter of the night, though, a few specimens were collected just after dusk or during dawn. It was arhythmic during December. Present findings are in agreement to those made in Pakistan[4]. How-

ever, there are conflicting reports about the biting habits of this species which may be due to seasonal variability in its feeding times[11-14].

An. fluviatilis was collected throughout the year except January. Biting commenced between 1900 and 2000 hrs and continued till dawn without any definite rhythm. During monsoon, 71 per cent of the specimens were collected in the first half with a peak between 2000-2100 hrs which is in conformity with the previous observations made in Madhya Pradesh[3]. Observations made in north Canara[15] indicate that feeding takes place mainly in the first part of the night and in Nilgiris[16], the species was observed to feed throughout the night and most of the activity being confined to the second and third quarters.

An. jamesii was encountered during February and December with one specimen in each month.

An. nigerrimus was collected throughout the year and was most prevalent during May and from September to November. It mostly fed before midnight and was markedly crepuscular from October to December. Little seasonal variation in biting times was observed with maximum activity taking place between 2100 and 2400 hrs. Except during July a small secondary peak at dawn was observed which agrees with the observations made in Pakistan[4], where it was observed to feed in the first quarter of the night.

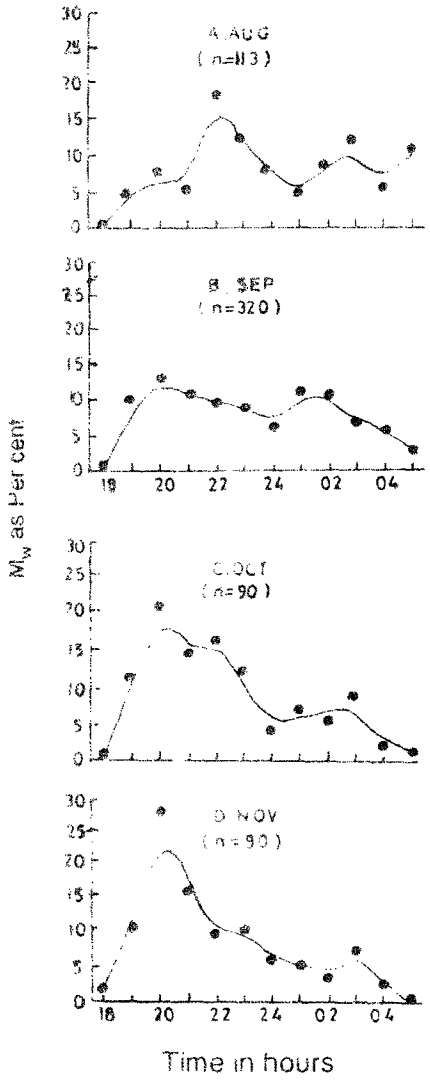

Fig. 4. Biting rhythms of *An. pallidus* during August-November

An. pallidus was most prevalent during monsoon and post-monsoon months. During winter it was crepuscular and peak biting was observed between 2000-2100 hrs with a small secondary peak just before dawn (Fig. 4). During August-October feeding took place from

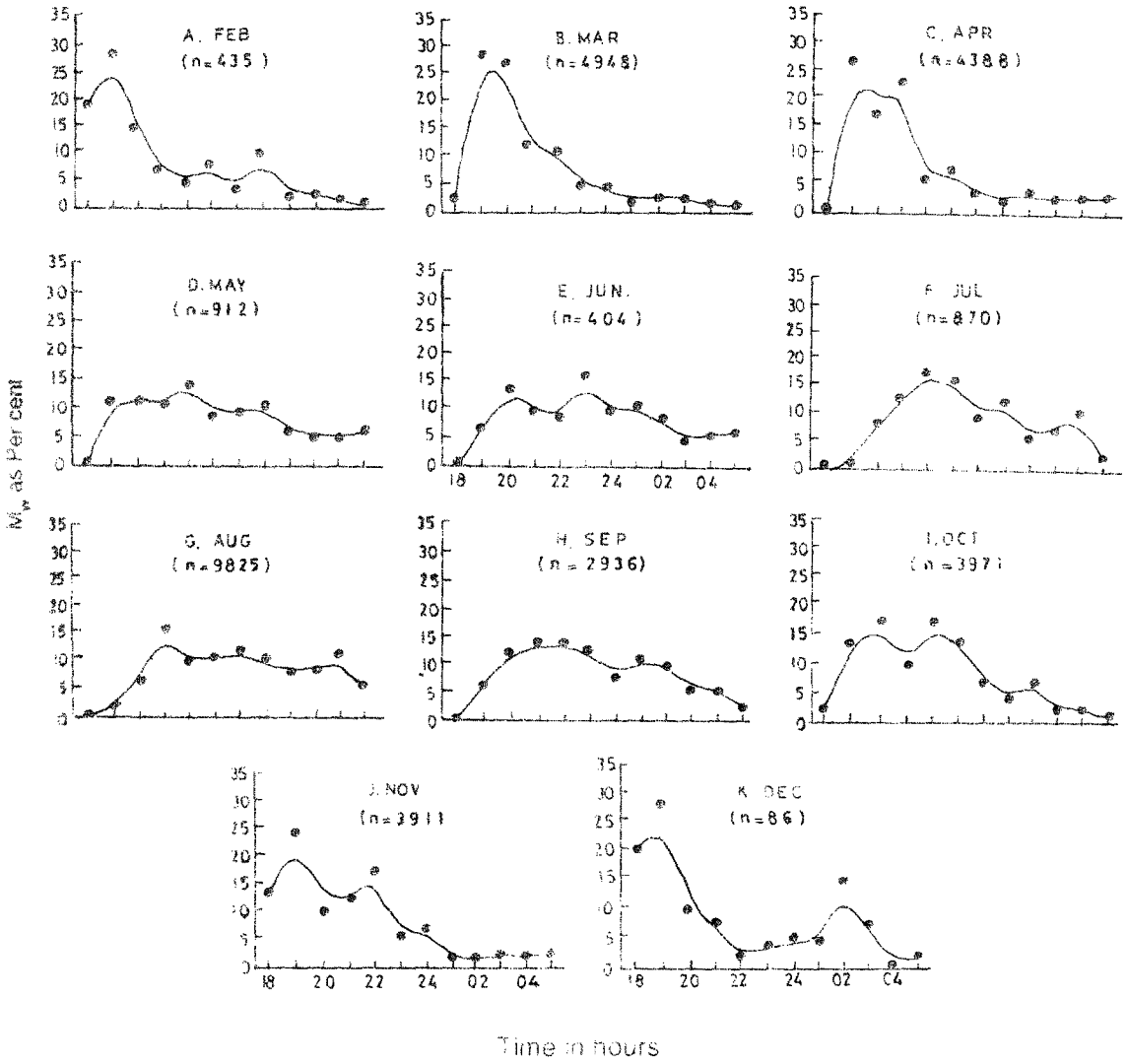

Fig. 5: Biting rhythms of *An. subpictus* during February-December

dusk-to-dawn with early and late night peaks. These observations are similar to those made in Tamil Nadu[2].

An. splendidus was collected frequently except during May, June, August and November. A total of 30 specimens were collected with maximum of eight during February and September. It essentially fed before midnight and maximum collection was made between 2000-2100 hrs which is in close agreement with the observations made in Madhya Pradesh[3].

An. stephensi was most abundant from February to April (131 out of 174) and was not encountered only during July,

October and December. Biting was mostly before midnight and maximum activity took place in the first quarter of the night. Biting continued till third and fourth quarters, though at a very low rate. Our results, in general agree with that reported from Pakistan[4].

An. subpictus was the most abundant species and was encountered in less numbers only during colder months. It was essentially a crepuscular feeder during cold season and maximum feeding occurred between 1800-1900 hrs (Fig. 5). During March-April maximum biting was observed between 1900-2000 hrs which further shifted towards midnight during May-July. From August onwards shift in the peak biting activity towards early hours of the night was observed. From May to August it was active throughout the night with enhanced activity at pre-dawn hours. The present curves comply with observations made in Pakistan[4], but differ slightly from the biting rhythms observed in Tamil Nadu[2], where maximum biting was reported between 2100-2200 hrs. Present findings also differ from the observations made in Pattukottai area[17] and Madhya Pradesh[3], where most feeding was observed after midnight.

An. tessellatus was collected throughout the year except in January. During April-May maximum biting took place in the first half of the night which shifted to the third quarter during September-October (Fig. 6). Biting continued till dawn or pre-dawn hours during these months. Very little information is available about its night biting activity in India.

An. theobaldi was collected only during February and August. Biting was observed between 2000-2300 hrs in February (a total of three specimens) and one specimen was collected biting between 0200-0300 hrs in August. Present observations agree with those made in Madhya Pradesh[3].

An. turkhudi was collected during February (one specimen), March and April

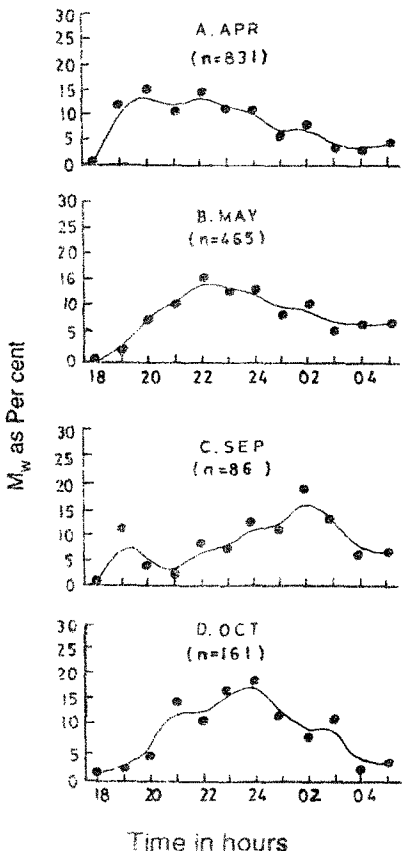

Fig. 6: Biting rhythms of *An. tessellatus* during April-May and September-October

(a total of 65 specimens). It exhibited multimodal biting rhythm with maximum activity before midnight, though it continued till dawn during March. No information is available on its night biting activity in India.

An. vagus was prevalent during monsoon season only and collected infrequently in July and September (a total of seven specimens). In the month of August (17 specimens), maximum biting took place before midnight with peak between 2000-2200 hrs. In Myanmar maximum activity was observed at about midnight[9].

An. varuna was prevalent throughout the year. It exhibited marked seasonal shift in its biting activity (Fig. 7). During summer maximum biting was observed in the second quarter of the night which shifted to the third in rainy season. In November, the shift towards the first half of the night was clearly evident. Although, peak biting activity shifted between different quarters, it took place throughout the night with a small increase in activity at dawn except during winter. In Madhya Pradesh[3] maximum biting on human and bovine baits was observed between 1800 and 2100 hrs.

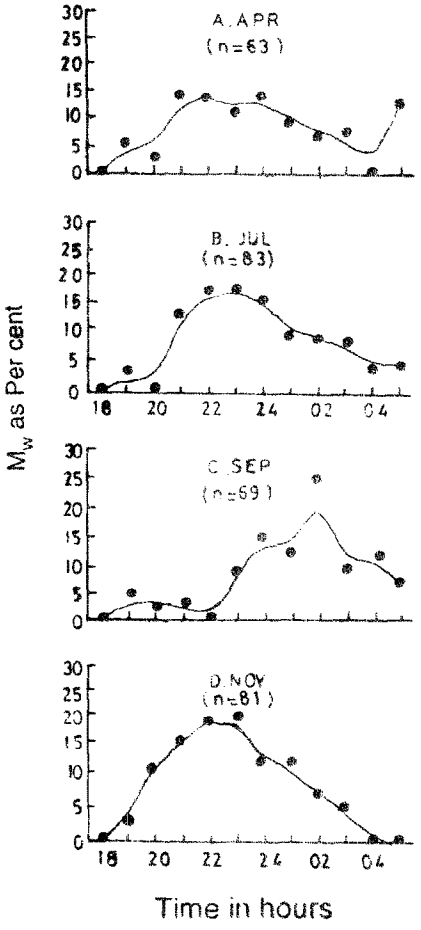

Fig. 7: Biting rhythms of *An. varuna* during April, July, September and November

On the basis of trends observed in the biting curves of anophelines in Kheda district, their nocturnal behaviour could be classified as: Unimodal — *An. vagus, An. stephensi, An. varuna, An. culicifacies* and *An. subpictus;* Bimodal — *An. pallidus;* and Multimodal — *An. turkhudi, An. tessellatus, An. fluviatilis, An. aconitus,* and usually *An. annularis, An. nigerrimus* and *An. barbirostris.*

Little information is available on the physical and genetic factors influencing the biting behaviour and rhythms of mosquitoes. It has been suggested[18] that a mosquito may be restrained from biting by unfavourable microclimatic conditions or unfavourable local weather

conditions. Various species of culicine bite (in nature) at a particular phase in the ovarian cycle indicating a possible relationship between feeding activity and the ovarian development, though microclimate and weather may also act important restraining factors. The possibility of the involvement of genetic factors has been postulated which could influence nocturnal behaviour on the basis of genetic causes associated with seasonal shift in the degree of anthropophilism, endophagy and endophily[4]. Further research in the field of species complexes and ovipositional behaviour may provide more convincing explanation for the seasonal shift in the biting activity of anophelines.

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Domestic Breeding Sources and their Contribution in *Anopheles stephensi* Breeding in Dindigul, Tamil Nadu

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Longitudinal study taken up for one year in 10 different types of breeding habitats in Dindigul town, Tamil Nadu, revealed that out of 51,785 habitats 225 (0.43%) were found positive for *Anopheles stephensi* immatures. The overall positivity varied between 0.03 to 1.31% with peak density during July. The observed habitat-wise positivity was overhead tanks 0-7.07%; wells 0-1.69%; under ground tanks 0-2.26%; tappits 0-2.36%; outside tanks (permanent) 0-2.42%; outside tanks (temporary) 0-0.39%; inside tanks (permanent) 0-2.0%; inside tanks (temporary) 0-3.6%; barrels 0-1.32% and others 0-25.0%. In 16.0% habitats *An. stephensi* was found breeding with *An. subpictus*, *Aedes aegypti*, *Ae. vittatus* and *Culex quinquefasciatus* in different combinations. Overhead tanks were found to contribute maximum *An. stephensi* breeding in this area.

Keywords: *Anopheles stephensi*, Domestic breeding sources, Tamil Nadu

INTRODUCTION

Anopheles stephensi is a well established urban vector of malaria in India. The species has been incriminated in various parts of India and is found very efficient vector in urban areas[1-4] and in certain periurban areas in association with *An. culicifacies*[5]. The species breeds in a variety of permanent, semi-permanent and temporary domestic and peri-domestic habitats. It has been observed to breed mainly in wells and cisterns in Salem (Tamil Nadu)[6], Hy-

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derabad (Andhra Pradesh)[7]. During a longitudinal study in Panaji, Goa, this species has been found to breed in various habitats and the densities varied during different seasons[8].

Anopheles stephensi contributes about 70% of the total malaria cases reported every year from Tamil Nadu. The knowledge of breeding of this species in various habitats during different seasons become very essential for understanding the variations in the incidence of malaria and for organising antilarval measures. The transmission of malaria in Dindigul is perennial with peak transmission between June and October. Therefore, a longitudinal study was taken up on *Anopheles stephensi* immature population in Dindigul reporting about 10,184 cases with an API of 54.24 during 1993. The results of the study are presented in this paper.

MATERIALS AND METHODS

Study area

Dindigul is situated between 10-11°S latitude and 78-79°E longitude with an area of 14.01 sq km and a population of 1.82 lakhs (1991 census). The town is divided into 44 wards. Topographically the land is slightly undulating with low subsoil water. It has a tropical climate with pleasant weather throughout the year, with temperature and humidity ranging from 16.8-39.3°C and 36.2-89.4% respectively. Dindigul receives both northeast and southwest monsoons with an average rainfall of about 900 mm every year starting from the month of May till December. The study was carried out in all the 44 municipal wards between June 1994 to May 1995.

Sampling was carried out in all the probable breeding sources totaling 51,785 in numbers, which included 3317 overhead tanks, 1654 wells, 1872 underground cement tanks, 1404 tappits (underground tanks used for collecting drinking water supply), 5107 permanent and 26,052 temporary outside cement tanks/cisterns, 2586 permanent and 3828 temporary inside cement tanks/cisterns, 5945 barrels and 20 other peri-domestic sources. The total number of sources checked and number found breeding during a month was pooled together to find the breeding percentage of different sources.

Five samples were taken from each well with the help of a well net (10 inch diam). Similar sampling was done at other breeding sites with the help of a dipper (150 ml capacity, 6 cm diam). All the immatures collected were transported to the laboratory and reared at room temperature. Dog biscuits and yeast in ratio of 3:1 was provided as larval food. The larvae and emerged adults were identified using keys of Christophers[9], Barraud[10] and Puri[11].

RESULTS AND DISCUSSION

Out of 51,785 habitats checked, 1205 (2.32%) were found positive for mosquito breeding. *Anopheles stephensi*

emerged from 225 (18.67%) of the total positive habitats. The rest 980 (81.33%) were positive for other mosquito species.

A total of 11 species of mosquitoes emerged from the immature samples collected from 1205 positive breeding sources. They were *Anopheles stephensi* (18.6%), *An. subpictus* (3.6%), *Ae. aegypti* (61.2%), *Ae. vittatus* (0.3%), *Ae. albopictus* (0.1%), *Cx. quinquefasciatus* (15.6%), *Cx. gelidus* (0.1%), *Cx. tritaeniorhynchus* (0.2%), *Cx. (lutzia) fuscanus* (0.1%), *Cx. whitmorei* (0.1%) and *Armigeres subalbatus* (0.1%).

When the positive index for the total breeding sites were taken into consideration it is observed that the breeding index varied between 0.67 and 3.91% during various months with peak density in November. *An. stephensi* index varied between 0.03% during April and 1.31% during July respectively. The habitat-wise breeding is represented in Table 1. The data shows that overhead tanks support maximum *An. stephensi* breeding ranging between 0.58 and 7.07% during April and September respectively. The overall positive index varied between 1.16% during April and 7.6% in September. It is interesting to note that 81.5% of the total overhead tanks found positive were breeding *An. stephensi*.

In case of wells *An. stephensi* breeding was observed in the months of June (0.58%), July (1.69%), October (1.37%) and November (0.65%). The low density in wells may be attributed to the stocking of larvivorous fish *Gambusia affinis*. In underground tanks breeding could be observed throughout the year with 54.4 and 75% showing breeding of *An. stephensi* during July and December respectively.

The tappits used for storing drinking water are normally kept open. These accounted for higher breeding of *An. stephensi* during July, September and October with 2.36, 1.66 and 1.39% respectively. This may have been supported by the conducive micro-environmental condition present in these type of habitats.

Interestingly, breeding was observed throughout the year in both outside and inside tanks. Though most of them were found with *Ae. aegypti* breeding. Temporary outside tanks accounted for higher breeding, with more *An. stephensi* breeding accounted during June to December ranging from 3.2-15.7%. In case of permanent outside tanks 0.48% of the habitats were found breeding *An. stephensi*. The other breeding sources like barrels, grinding stones etc. were found breeding during July to October corresponding with the rainy season.

An. stephensi was found breeding in association with other mosquito species in about 16% of the positive *An. stephensi* habitats. *An. subpictus* was associated in 0.9%. *Cx. quinquefasciatus* in 3.1%, *Ae. aegypti* in 8.9% and *Ae. vittatus* in 1.3%. In four habi-

Table 1. Habitat-wise breeding of *Anopheles stephensi* in Dindigul town

Months (1994-95)	Total breeding sites			Domestic breeding sources					
	A	B	C	OHT			Well		
				A	B	C	A	B	C
Jun	4046	130 (3.2)	13 (0.32)	414	15 (3.62)	11 (2.66)	172	1 (0.58)	1 (0.58)
Jul	6290	227 (3.61)	83 (1.31)	515	36 (5.85)	34 (15.53)	178	6 (3.37)	3 (1.69)
Aug	4287	75 (1.74)	13 (0.3)	217	6 (2.76)	4 (1.84)	109	1 (0.92)	0
Sep	3562	99 (2.77)	25 (0.7)	198	15 (7.57)	14 (7.07)	154	0	0
Oct	3812	78 (2.04)	31 (0.81)	385	13 (3.38)	11 (2.86)	146	4 (2.71)	2 (1.37)
Nov	4577	179 (3.91)	13 (0.28)	178	12 (6.74)	5 (2.81)	178	4 (2.25)	1 (0.56)
Dec	4118	97 (2.36)	19 (0.46)	438	13 (2.97)	12 (2.74)	165	3 (1.82)	0
Jan	3274	76 (2.32)	8 (0.24)	98	8 (8.1)	6 (6.12)	29	0	0
Feb	5312	57 (1.07)	4 (0.08)	329	4 (1.22)	4 (1.22)	167	2 (1.20)	0
Mar	3169	74 (3.34)	3 (0.09)	160	0	0	123	1 (0.81)	0
Apr	5201	35 (0.67)	2 (0.03)	172	2 (1.16)	1 (0.58)	144	1 (0.69)	0
May	4137	78 (1.89)	11 (0.27)	113	6 (5.31)	4 (3.54)	89	1 (1.12)	0
Total	51,785	1205 (2.32)	225 (0.43)	3317	130 (3.91)	106 (3.19)	1654	24 (1.45)	7 (0.42)

contd...

Table 1. (*contd.*)

Months (1994-95)	Domestic breeding sources					
	UGT			Tappit		
	A	B	C	A	B	C
Jun	416	19 (4.57)	1 (0.24)	149	12 (8.05)	0
Jul	756	22 (2.91)	12 (1.59)	296	14 (4.73)	7 (2.36)
Aug	77	6 (7.79)	0	96	1 (1.04)	0
Sep	69	3 (4.34)	0	60	1 (1.66)	1 (1.66)
Oct	127	9 (7.09)	2 (1.57)	72	2 (2.78)	1 (1.39)
Nov	64	1 (1.56)	0	121	17 (14.05)	0
Dec	133	4 (3.00)	3 (2.26)	176	5 (2.84)	0
Jan	33	2 (6.06)	0	178	8 (4.49)	0
Feb	80	2 (2.50)	0	97	2 (2.06)	0
Mar	19	1 (5.26)	0	37	0	0
Apr	80	2 (2.5)	0	57	3 (5.26)	0
May	18	2 (11.11)	0	15	3 (20.0)	0
Total	1872	73 (3.89)	18 (0.96)	1404	68 (4.84)	9 (0.64)

contd...

Table 1. (*contd.*)

Months (1994-95)	Peri-domestic breeding sources								
	OST (P)			OST (T)			IST (P)		
	A	B	C	A	B	C	A	B	C
Jun	328	5 (1.52)	0	1797	62 (3.45)	3 (0.17)	175	2 (1.14)	0
Jul	619	38 (6.14)	15 (2.42)	2419	75 (3.10)	9 (0.37)	228	10 (4.39)	1 (0.44)
Aug	94	7 (7.45)	1 (1.06)	3500	38 (1.09)	6 (0.17)	50	4 (8.0)	1 (2.0)
Sep	283	12 (4.24)	0	1529	40 (2.61)	5 (0.32)	269	11 (4.08)	1 (0.37)
Oct	426	14 (3.29)	4 (0.94)	1794	22 (1.23)	7 (0.39)	217	4 (1.84)	2 (0.92)
Nov	553	11 (1.99)	0	2579	99 (3.84)	6 (0.23)	210	11 (5.24)	0
Dec	359	16 (4.46)	0	1873	42 (2.24)	4 (0.21)	251	6 (2.39)	0
Jan	222	5 (2.25)	0	2072	36 (1.73)	0	112	10 (8.92)	2 (1.78)
Feb	557	8 (1.44)	0	2437	23 (0.94)	0	402	7 (1.74)	0
Mar	521	7 (1.34)	1 (0.19)	1473	54 (3.67)	1 (0.07)	140	2 (1.43)	1 (0.71)
Apr	430	2 (0.47)	0	2133	11 (0.52)	0	394	3 (0.76)	0
May	715	27 (3.78)	4 (0.56)	2446	36 (1.47)	2 (0.08)	138	1 (0.72)	0
Total	5107	152 (2.97)	25 (0.48)	26,052	538 (2.06)	43 (0.16)	2586	71 (2.74)	8 (0.30)

contd...

Table 1. (*contd.*)

Months (1994-95)	Peri-domestic breeding sources								
	IST (T)			Barrels			Others		
	A	B	C	A	B	C	A	B	C
Jun	300	7 (2.33)	0	295	7 (2.37)	0	0	0	0
Jul	448	18 (4.02)	0	731	8 (1.09)	2 (0.27)	0	0	0
Aug	68	8 (11.76)	0	76	4 (5.26)	1 (1.32)	0	0	0
Sep	305	12 (3.93)	1 (3.6)	685	4 (0.58)	2 (0.29)	10	1 (10.0)	1 (10.0)
Oct	218	1 (0.46)	0	421	5 (1.19)	1 (0.24)	6	4 (66.66)	1 (16.66)
Nov	330	17 (5.15)	1 (0.30)	364	7 (1.92)	0	0	0	0
Dec	417	5 (1.20)	0	306	3 (0.98)	0	0	0	0
Jan	214	4 (1.86)	0	316	3 (0.94)	0	0	0	0
Feb	471	9 (1.91)	0	772	0	0	0	0	0
Mar	292	8 (2.74)	0	354	1 (0.28)	0	0	0	0
Apr	520	6 (1.15)	0	1267	3 (0.24)	0	4	2 (50.0)	1 (25.0)
May	245	1 (0.41)	0	358	1 (0.28)	1 (0.28)	0	0	0
Total	3828	1 (2.50)	0 (0.05)	5945	46 (0.77)	7 (0.11)	20	7 (35.0)	3 (15.0)

OHT — Overhead tanks; UGT — Underground tanks; OST (P) — Outside cement tanks (Permanent); OST (T) — Outside cement tanks (Temporary); IST (P) — Inside cement tanks (Permanent); IST (T) — Inside cement tanks (Temporary); A — No. surveyed; B — No. positive for breeding (%); C — *An. stephensi* breeding (%).

Table 2. *An. stephensi* breeding in association with other mosquito species

No. of breeding sites				No. of samples in which *An. stephensi* was found breeding with							
Surveyed	No. (+)ve	*An. ste.*	*An. ste.* + other sp	*An. sub.*	*Cx. quin.*	*Ae. aegy.*	*Ae. vitt.*	*Cx. quin.+Ae. aegy.*	*An. sub.+Ae. aegy.*	*Cx. quin.+Ae. aegy.+Ae. vitt.*	*An. sub.+Cx. quin.+Ae. aegy.*
51,785	1205	189	36	2	7	20	3	1	1	1	1

An. ste. — Anopheles stephensi; An. sub. — Anopheles subpictus; Cx. quin. — Culex quinquefasciatus; Ae. aegy. — Aedes aegypti and *Ae. vitt. — Aedes vittatus.*

tats it was associated with *An. subpictus, Cx. quinquefasciatus, Ae. aegypti* and *Ae. vittatus* in different combinations (Table 2).

An. stephensi is found to breed throughout the year, with an increasing trend from May and reaching its peak during July. This is followed by a decline during August and picking up from September. From November till April the declining trend continues. During this period the increase in the number of peri-domestic secondary foci (like the terrace water collection, water collection in the smaller discarded tins, coconut shells, etc.) enhanced the breeding potential of *An. stephensi* in this area. Interestingly, though there is an increase in mosquito breeding habitats during November, the *An. stephensi* breeding declined. During the dry season, the mother foci of *An. stephensi* might have been maintained in the ground level cisterns like tappits, outside tanks and sumps.

The study indicated that *An. stephensi* could be found breeding in various habitats during different seasons. In most habitats this species was breeding in various proportions during different months. This study indicated that overhead and underground tanks are the major breeding sources for *An. stephensi*. Though wells are supposed to be one of the major sources of breeding, it could easily be controlled by bioenvironmental control measures[12] using larvivorous fish. Therefore, it is suggested that greater attention should be given to overhead tanks, underground tanks and tappits for an effective control of this vector in Dindigul town. The mosquito proofing of these sources with the cooperation of Government agencies and community would be rewarding for the long-lasting control of both vector and malaria in this area.

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Socio-economic Aspects of Malaria in Kheda District, Gujarat

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Study on the socio-cultural factors and economic loss due to malaria in rural and urban areas of Kheda district, Gujarat revealed that factors such as education, profession, income, housing pattern, social groups, water storage and treatment seeking behaviour play an important role in malaria transmission. However, the difference of these components in respect to malaria cases between rural and urban areas were statistically insignificant. Mean monetary loss per malaria episode was found to be more in urban area (Rs. 393.59) as compared to rural (Rs. 157.59). The study also suggests the importance of socio-cultural factors in implementing any community health programme. Health education is needed to make the people aware and health conscious for prevention of disease at their own level.

Keywords: Economic loss, Malaria, Mosquito, Socio-economic factors

INTRODUCTION

Malaria continues to be one of the major public health problem in India and many other countries. Recent outbreak of malaria in Rajasthan, Assam, and other northeastern states indicate the severity of the problem despite continuous control efforts. The complexity and prevalence of the disease is governed by multiple constraints as technical, operational and financial[1] and thus go on inflicting heavy social and economic losses to mankind. It not

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only causes sickness and individual agony but also exerts extra burden on the government. Economic loss due to malaria at the time of independence was estimated about Rs. 7500 million a year[2]. Socio-economic conditions of a community are directly related with the problem of malaria. Low socio-economic status of the people contributes in creating source and spread of malaria and hinders disease control strategy[3]. It has been reported earlier that the prevalence, incidence and transmission of malaria is associated with the low income of the family, poor housing, deficient personal hygiene, low literacy rate, poverty and outdoor activities after dusk[4].

There are several other socio-cultural factors ranging from those operating at the individual level to those at the community level which are responsible for the prevalence of the disease. The magnitude of the problem differs from place-to-place due to variation in mosquito breeding conditions, dwelling structures and other developmental activities. The present study was designed to find out some of the socio-cultural factors which determine the prevalence of malaria and an attempt was made to assess the economic loss per malaria episode in a rural and an urban area of Nadiad taluka, Kheda during October 1993 to December 1994.

MATERIALS AND METHODS

For the purpose of the study, seven villages of Nadiad taluka namely, Tundel, Pij, Akhadol, Keriavi, Narsanda, Rajnagar and Kanjoda were selected on the basis of annual parasite incidence (API) of last two years. A total of 100 respondents from above mentioned villages and 50 respondents from Nadiad town were selected randomly from a list of confirmed malaria cases. Blood smears of all respondents who had suffered from malaria during last one year were examined either at respective primary health centres (PHCs) or at the malaria clinic of Malaria Research Centre (MRC). The schedule contained questions regarding family background, housing pattern, water supply/storage, surrounding hygiene, general health problems, knowledge about malaria and its treatment, level of health education, personal protection methods practised and cost incurred on each malaria episode. The respondents were interviewed at their houses and schedule were filled up. Apart from collecting background information about each sick person, assessment of the expenditure which the family had incurred on medication, transportation and special diet, if any, was also made. Indirect cost like wage loss due to sickness was also calculated on the basis of information collected. The loss incurred on each component was determined by taking means as per standard technique. Data obtained were later processed and analysed statistically.

RESULTS AND DISCUSSION

Salient features of the study such as mean age of the respondent, occupants per house, literacy rate, mean annual

income, per capita income, mean mandays lost and mean total loss per case are shown in Table 1. The mean age of the respondents and occupants per house in two areas remained more or less same. Whereas, literacy rate annual income, per capita income and mean mandays loss were comparatively higher in the urban area, due to better facilities and job opportunities. High values of standard error in the mean annual income of respondents and households indicated wide variation in their income.

Age- and sex-wise distribution of the respondents show that age group of 15-40 yr suffers more from malaria (>50%) in both the areas, the reason may be more exposure of such population due to their diversified activities for livelihood/studies/business etc. Sex-wise analysis of the data from both the areas shows that males are more at risk in comparison to females, may be because the latter are better clothed, less mobile and usually remain involved in indoor activities. In rural area 80% of the respondents were either illiterate or had education up to primary level only and contributed maximum malaria positive cases, whereas the higher literacy rate was noticed in urban area (44%). Although literacy rate is more in urban area, there is increase in malaria cases thereby indicating that education alone is not all to control malaria (Table 2). Banguero[5] also did not find any association between the education level and the disease incidence in Columbia. Analysis of respondents in respect to their profession, income and social groups is given in Table 3. It was found that dependents (39.33%) and labourers (33.33%) suffer more from malaria. In rural area particu-

Table 1. Salient features of respondents

Attributes	Rural (n = 100)	Urban (n = 50)
Mean age (yr)		
Respondent	26.8±1.4*	28.6±11.9
Households	25.5±0.7	24.3±0.9
Mean persons/house	4.8±0.1	5.1±0.2
Literacy rate (%)	61.7	67.6
Mean annual income (Rs.)		
Respondents	3172.77±323.52	8755.28±1471.0
Households	12166.19±1141.12	30480.00±4089.75
Per capita income (Rs.)	2545.22±150.30	5953.12±2680.57

*Mean ±SE.

Table 2. Per cent distribution of respondents by sex, age and literacy as per their place of residence

Area	Sex*		Age**			Education+			
	Male	Female	<14	15-40	>40	Illi-trate	Pri-mary	Sec-ondary	Grad-uate
Rural (n=100)	60	40	20	62	18	33	47	20	–
Urban (n=50)	56	44	22	58	20	28	28	38	6
Mean	58.6	41.3	20.6	60.6	18.6	31.3	40.6	26.0	2.0

*t-3.571, df-1, p>0.05; **t-2.039, df-2, p>0.05; +t-1.498, df-3, p>0.05.

Table 3. Per cent distribution of respondents in respect of profession, income and social groups

Area	Profession*					Annual income** (Rs. in ,000)				Social groups+		
	Far-mers	Lab-ourers	Ser-vice	Busi-ness	Depen-dents	<10	10-30	30-50	Depen-dents	SC	OBC	Other castes
Rural (n=100)	8	42	–	13	37	58	5	0	37	15	69	16
Urban (n=50)	2	16	26	12	44	24	24	8	44	36	52	12
Mean	6.0	33.3	8.6	12.6	39.3	46.6	11.3	2.6	39.3	22.0	63.3	14.6

*t-1.319, df-4, p>0.05; **t-1.026, df-3, p>0.05; +t-1.217, df-2, p>0.05.

larly the labour population is at higher risk of malaria due to their outdoor sleeping habits, sleeping without mosquito nets or any other protective devices, frequent movement and inadequate treatment. Similar observations were also made in studies carried out elsewhere[6-9]. However, *t*-test did not reveal any significant difference in the distribution of malaria cases between rural and urban areas. In both the areas 58% malaria cases were in lower income group, it may be due to unhygienic living conditions and lack of knowledge regarding the disease. Pinikahana[10] found that insufficient knowledge of malaria among the people played a major role in high malaria transmission in an irrigation project in Sri Lanka. Whereas, Mata[4] reported poor housing and deficient personal hygiene as one of the most important socio-cultural factors responsible for malaria transmission. Maximum num-

ber of positive cases were from other backward classes in both the areas, whereas socio-economically upper castes had very low malaria incidence. However, there was no significant difference in the distribution of malaria cases between rural and urban area in respect to these attributes ($p>0.05$).

Dwelling details of the respondents indicate that in rural area about 80% of the houses are mud-plastered in contrast to urban area, where 78% houses are either partly or completely cemented. The mud houses are often mentioned as a drawback in the conventional malaria control programme using insecticides because of the practice of mud-plastering soon after spray[11]. It appears that houses with only one or two rooms and one door, contributes more malaria cases, in contrast to those with more rooms and doors (Table 4). In rural area >75% houses had no ventilation and contributed substantial malaria cases due to darkness and provide better resting places for mosquitoes thereby increasing the possibility of man-mosquito contact. In urban area there was no significant association of the occurrence of malaria with ventilation. Benguero[5] also did not find any relationship between the degree of completion of house (roof, walls, windows, doors) and malaria incidence. Similarly, there was no correlation between separate kitchen and number of malaria cases in both the areas. The population living in close association with cattle (mixed

Table 4. Per cent dwelling details of respondents

Houses (150)	Area	%	Rooms			Doors			Windows	Ventilator	Separate kitchen	Mixed dwelling
			1	2	3+	1	2	3+				
Mud plastered	R	57	34	18	5	51	6	0	6	4	9	15
	U	22	18	2	2	16	4	2	22	4	4	0
Partly cemented	R	23	4	15	4	15	7	1	10	6	12	8
	U	28	22	6	0	24	4	0	28	16	4	4
Pucca	R	20	2	8	10	7	1	6	15	13	15	3
	U	50	12	18	20	14	12	24	50	34	36	4
Total	R	100	40	41	19	73	20	7	21	23	36	26
	U	100	52	26	22	54	20	26	100	54	44	8

R — Rural (n = 100); U — Urban (n = 50).

Table 5. Source of water supply, storage and surrounding hygiene

Water supply	%		Method of storage	%		Surrounding	%	
	R	U		R	U		R	U
Piped water	72	92	Buckets	31	30	Hygienic	81	64
			Drums	3	4			
			Tanks	6	28	Unhygienic	19	36
Well	28	8	Cisterns	60	38			

R — Rural (n = 100); U — Urban (n = 50); χ^2–5.18, df–1, $p<0.05$.

dwelling) showed less incidence of malaria due to zooprophylaxis of vector mosquitoes as both vectors *An. culicifacies* and *An. stephensi* predominantly bite cattle[12]. This trend is more apparent in rural areas in comparison to urban areas as cattle population is usually scanty in urban areas.

Detail about the water supply, storage and surrounding hygiene in rural and urban areas and their relationship with the occurrence of malaria is given in Table 5. In both rural as well as urban areas most of the houses receive intermittent piped water supply due to which there is a tendency to store water in domestic containers particularly in cisterns, tanks and mud pots, which provide additional mosquito breeding potential and enhance risk of malaria if not properly covered. It was observed that water storage containers like cisterns were found more in rural area (60%), whereas, cisterns (38%) and tanks (28%) were more in urban area. In certain situations particularly in rural areas, in presence of numerous peridomestic mosquito breeding sources such as river, ponds, wells, canal, drains and rice fields, surrounding hygiene does not play any significant role in the transmission of malaria (19%), however, in absence of said habitats in urban area surrounding hygiene seems to play an important role in disease transmission (36%).

More than 55% respondents in both the areas were of the opinion that malaria fever comes at every alternate day. Delayed treatment seeking behaviour was observed in both the areas and very less number of patients took treatment on Day 0 which may be one of the important factors for morbidity. People usually take treatment after two days in both the areas. Maximum number of patients recovered from malaria on Day 4, whereas this time lag was more in urban area (>5 days). Early treatment was sought by respondents in rural area (mean days 2.4) than urban respondents (mean days 3.3) which resulted in early recovery from malaria in the former (Table 6).

In rural area there is a regular surveillance mechanism under the primary health care system and therefore most

Table 6. Knowledge on occurrence of fever, treatment and recovery

Ocurrence of fever	%		Treatment taken on Day	%		Recovered from malaria (Day)	%	
	R	U		R	U		R	U
Daily	44	34	0	12	14	1	1	2
			1	8	16	2	18	4
Alternate day	56	66	2	44	24	4	42	18
			3	18	22	>5	39	76
			5+	18	24			

R — Rural (n = 100); U — Urban (n = 50); χ^2–1.38, df–1, p>0.05; χ^2–6.55, df–4, p>0.05; χ^2–19.22, df–1, p<0.001.

Table 7. Economic loss* due to malaria

Value (Rs.)	Rural	Urban	*t*-value
Loss in wages			
Patient	65.85±7.4	81.52±26.2	3.306, p<0.01
Attendent	29.85±3.3	19.90±6.0	2.219, p<0.01
Expenses on			
Treatment	43.73±9.2	235.24±76.2	1.909, p<0.05
Travel	2.80±1.1	13.04±2.7	0.696, p>0.05
Special diet	9.10±3.2	36.69±9.9	0.038, p>0.05
Other loss	6.64±1.2	7.20±1.0	2.146, p<0.05
Total loss per episode	157.97±25.4	393.59±122.04	6.420, p<0.01
Mandays lost			
Patient	7.0±0.8	9.0±1.2	-
Attendent	5.0±0.4	5.0±1.1	-

*Mean±SE.

of the malaria cases are detected and treated in short duration and thus minimizes the expenditure on consultation, medicine, transport, laboratory test etc. In urban area, peripheral surveillance, practice of self medication, clinical diagnosis and inadequate treatment of the disease may be some of the reasons for the delayed recovery.

Economic loss on each malaria episode was calculated and is given in Table 7. Mean monetary loss due to

malaria was observed more than double in urban respondents (Rs. 393.59) as compared to rural (Rs. 157.97). Similarly, loss in wages, treatment, special diet and mean mandays loss of the patients were higher in urban area except loss in helper's wage due to less number of employed helpers in urban area (20%) as compared to rural area (66%). A significant difference was observed between rural and urban areas in break up of all the components except travelling and special diet. Sharma *et al.*[11] found average total expenditure of Rs. 73.0 on each malaria episode in the villages of Nadiad taluka during demonstration of bioenvironmental control of malaria. This was in comparison to that of Rs. 151.0 in control area. If these figures are multiplied by the total number of reported malaria cases in the country, the statistics may present a correct picture of losses incurred by the country on malaria. Ray[13] reported that economic loss to the community on account of loss of wages alone as per the population and the wage scale of that time would have reached a staggering level of Rs. 7350 million per year. He also reported that the average number of working days lost per man per year was about 15 days because of disability during primary attacks, relapse and re-infections. This was an extremely modest estimate as in hyper- and meso-endemic areas the number of sick days are usually more.

The present investigation suggests that consideration of socio-cultural factors is pre-requisite for practising any community health programme. Therefore, it is of paramount importance to disseminate the knowledge on prevention of malaria through health education at grass root level and make the people aware and health conscious so that they can take care of themselves by reducing the mosquito breeding sources and promptly getting their blood tested in case of fever. Such individual approach will pave way for community participation in disease vector control programme. When communities are involved the operational cost of control programme reduces, thus judicious resource utilization will result in all round prosperity and economic development of the country.

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SHORT NOTE

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Role of Biological Agents for the Control of Mosquito Breeding in Rice Fields

RAJNI KANT, S.D. PANDEY[a] and S.K. SHARMA

Keywords: *Azolla*, Biocide, Mosquitoes, Rice fields

Intensive agriculture coupled with ill-planned irrigation system, have changed the micro- and macro-climatic conditions which are responsible for the increased mosquitogenic potential and rise in the incidence of certain vector-borne diseases. Rice is one of the most important food crops and primary staple diet for many. It is cultivated on large-scale throughout India and area under paddy cultivation is expected to grow more due to increasing population. Vast surface area of rice fields and constant long standing water creates favourable breeding sites for many species of mosquitoes including some important vectors of malaria and Japanese encephalitis[1-5]. To control mosquito breeding in rice fields was the main agenda in post-resurgence period. Control of adult mosquitoes with residual insecticides has been unsatisfactory due to insecticide resistance, human refusals to spraying and environmental considerations. In view of this, an attempt was made to evaluate three biological agents namely, *Azolla*, *Poecilia reticulata* and biocide for the

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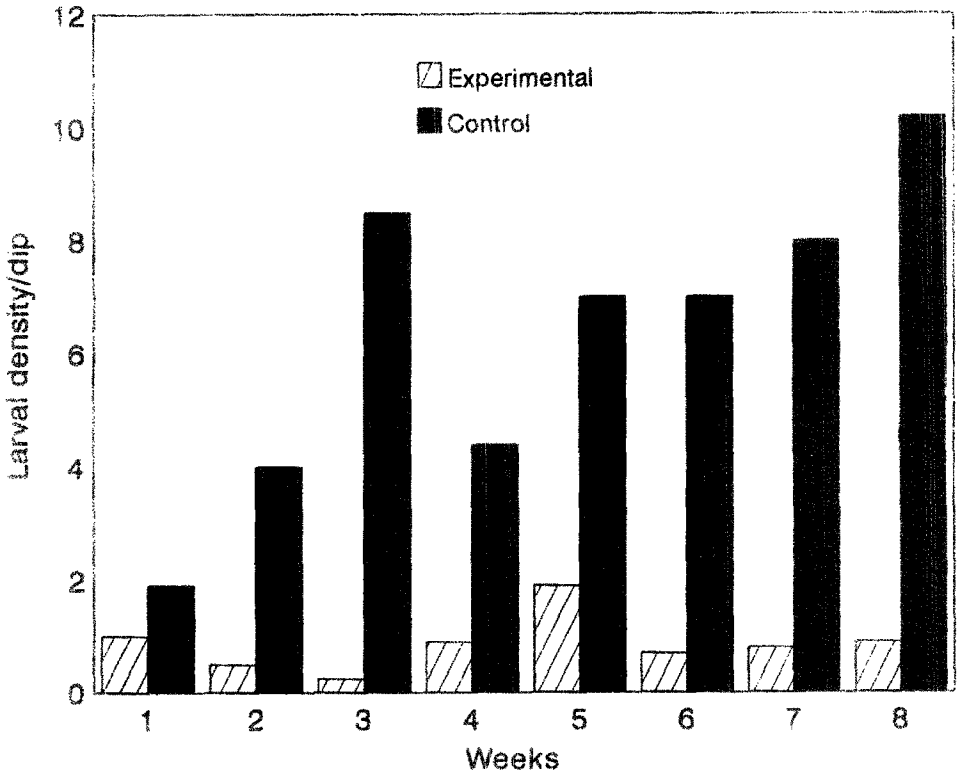

Fig. 1: Effect of *Azolla* on mosquito breeding

control of mosquito breeding in rice fields.

Small-scale field trials were conducted during 1989-90 in growing rice fields (vegetative growth phase of the crop) in a predominantly rice cultivating village of Kheda district, Gujarat. Rice fields selected were near the canal network, when breeding of *Anopheles culicifacies* and *An. subpictus* was high and plant height was around 20-40 cm. Due to seepage from canal, water depth in these fields remained more or less 10 cm and pH ranged between 7 and 7.5. Three biological agents namely, aquatic fern (*Azolla pinnata*), larvivorous fish (*Poecilia reticulata*) and biocide (*Bacillus sphaericus* 1593) were applied in three different rice fields of about 100 sq m area. *Azolla* was obtained from Gujarat Agricultural University, Anand and was applied @ 150 g/sq m (wet weight). Since the growth of *Azolla* is influenced by seasonal changes, its impact on mosquito breeding was observed in both monsoon as well as non-monsoon crops. *Poecilia reticulata* was collected from local ponds and was introduced in rice fields @ 5-10 fish/sq m. Formulation of *B. sphaericus* was obtained from Central Drug Research Institute, Lucknow and was applied @ 5 g/sq m in each rice field. The larval

and pupal density in all the fields was monitored by using a standard dipper (9.5 cm diam and 300 ml capacity). During the evaluation of biological agents in rice fields, no agrochemicals were applied. Fields treated with *Azolla* and larvivorous fish were monitored up to VIII and XII weeks respectively, whereas biocide treated plots were monitored on Days 0, 1, 2, 3, 7, 14, 21 and 28. Larval density was also monitored from three identical untreated control plots for comparison. Per cent reduction in larval density of biocide treated fields was calculated as per the formula of Mulla *et al*[6]. Results were analyzed statistically by applying student *t*-test.

Mosquito breeding in the rice agroecosystem of Kheda district and their seasonal abundance in relation to the height of plants have already been reported earlier[4,5]. Evaluation of biological agents for the control of mosquito breeding in rice fields showed that, *Azolla* application controlled mosquito breeding to a great extent (Fig. 1). Larval densities in *Azolla* treated rice fields were recorded below 2 larvae per dip, whereas in control fields densities remained high and fluctuated from 2-14 larvae per dip during monsoon and 0.6-12 larvae per dip during non-monsoon period (Table 1). Statistical analysis of data showed significantly lower larval densities in experimental fields than those in the control rice fields ($t = 3.79$ and 4.18; $df = 14$; $p<0.01$, for monsoon and non-monsoon crops, respectively). Thus, *Azolla* was quite effective in controlling the mosquito breeding in rice fields.

Slight increase in larval densities during monsoon in *Azolla* applied rice fields was due to intermittent rain which disturbed the layer of *Azolla*. Patches developed due to disturbance caused by the movement of insects and ani-

Table 1. Efficacy of *Azolla* on mosquito breeding in rice fields

Weeks	Monsoon crop (Larval density/dip)		Non-monsoon crop (Larval density/dip)	
	Experimental	Control	Experimental	Control
I	1.75	2.10	0.20	0.60
II	0.74	5.46	0.10	2.77
III	0.50	3.47	0.10	12.75
IV	0.93	5.10	0.45	3.65
V	1.84	6.60	0.60	7.40
VI	0.92	8.47	0.20	5.70
VII	1.29	5.62	0.07	10.40
VIII	1.60	14.84	0.10	6.00

$t = 3.79$ and 4.18, $df = 14$, $p < 0.01$ for monsoon and non-monsoon crops, respectively.

mals also facilitated oviposition resulting in increased mosquito breeding. The suppressing effect of *Azolla* on mosquito breeding has been reported earlier[7,8]. Besides providing mechanical barrier to mosquitoes for oviposition, it also act as an efficient biofertilizer because of the symbiotic relationship with blue green algae (*Anabaena azollae*) which fixes the atmospheric nitrogen[9].

After the introduction of *Poecilia reticulata* (guppy) in rice fields larval densities declined from 8 to 2 larvae per dip and was maintained up to 8 weeks. Thereafter, it increased because of the appearance of aquatic vegetation such as algae, *Marsilea*, *Ipomea* etc. which restricted the movement of the fishes. On the other hand the rice fields without fish had more or less the static larval densities with occasional fluctuations (Fig. 2). Statistical analysis also showed a significant difference in larval densities in experimental and control fields ($t = 3.87$, $df = 22$, $p < 0.01$).

Sharma *et al.*[10] found considerable reduction in the larval densities while using indigenous larvivorous fish in Kheda district, Gujarat. Nalim and

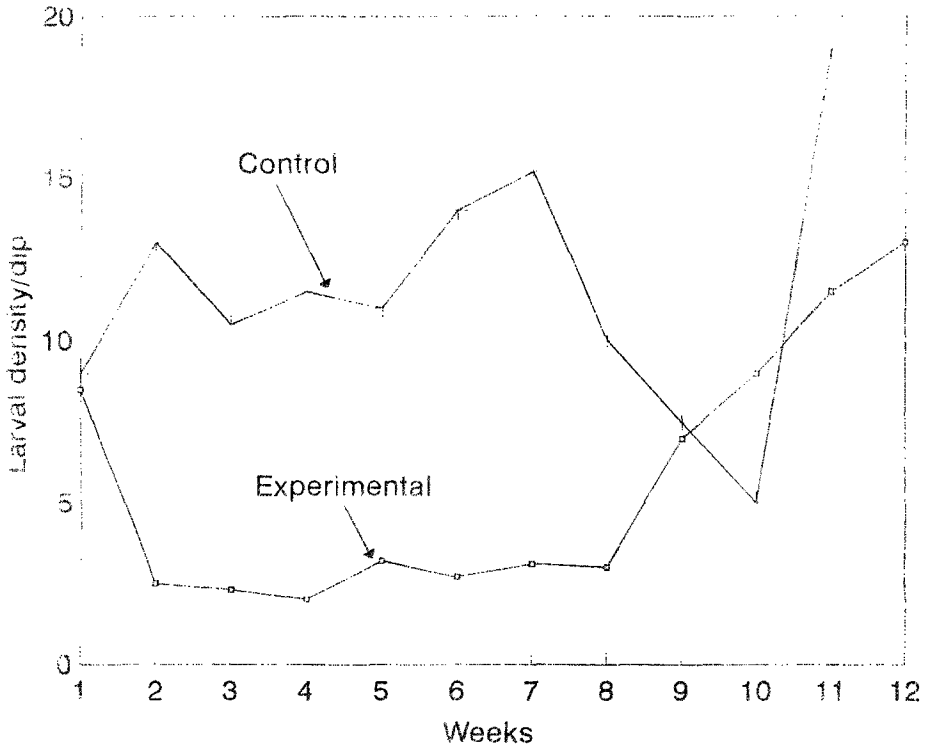

Fig. 2: Impact of *P. reticulata* on mosquito breeding in rice fields

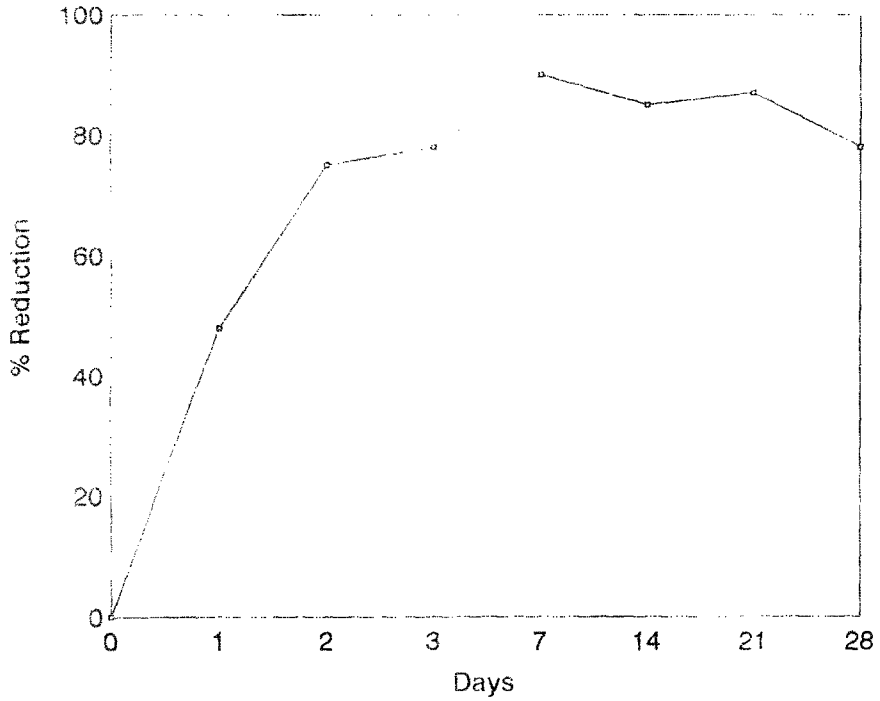

Fig. 3: Effect of *B. sphaericus* against mosquito larvae (III and IV instar)

Tribuwono[11] observed good efficacy of *P. reticulata* in paddy fields and a drastic decline in the number of malaria cases. Mosquito fish *Gambusia affinis* has also been evaluated to control mosquito breeding in rice fields[12].

Larval densities in biocide treated rice fields were reduced by 70% during the first week of application i.e., from 33.5 to 10.5 larvae per dip (Fig. 3). The reduction in subsequent weeks was slow, however there was no clear reduction in larval densities in control fields (Table 2). However the differences were statistically non-significant

Table 2. Efficacy of biocide (*B. sphaericus*) on mosquito breeding in rice fields

Days	Larval density/dip	
	Experimental	Control
0	33.50	9.40
1	15.50	8.40
2	14.50	16.10
3	13.37	17.35
7	10.50	24.15
14	9.90	17.70
21	6.10	11.65
28	5.25	5.85

$t = 0.06$, $df = 14$, $p > 0.05$.

($t = 0.06$, $df = 14$, $p > 0.05$). Although the larvicidal activity of *B. sphaericus* and *B. thuringiensis*[13] has been demonstrated earlier, their efficacy seems to be governed more by the prevailing conditions of the habitat and varies from strain to strain and type of formulation.

Thus, under optimum field conditions, all three agents namely, *Azolla*, larvivorous fish and biocide were found quite effective in suppressing the mosquito larval densities. However, biological agents have certain limitations eg., *Azolla* cannot withstand high temperature and starts dying as summer approaches. Receding water level results in the formation of patches in the *Azolla* layer which provide support for mosquito proliferation, however on further flooding it tends to regain its efficacy by forming a uniform layer in few days if water level is maintained. In fields with rapid drying up conditions the use of fish may not produce the desired results as formation of pool, patches and puddles creates problems for their survival and movement. Appearance of thick aquatic vegetation on water surface in later part of rice crop also restricts movement of fish and thereby the predation of mosquito larvae. Efficacy of biocide is also influenced by several abiotic and biotic factors such as organic and inorganic pollutants, surface vegetation, water depth etc. of the habitat and varies in different cases. Therefore, large-scale trials under different ecological situations with greater geographical reconnaissance are needed.

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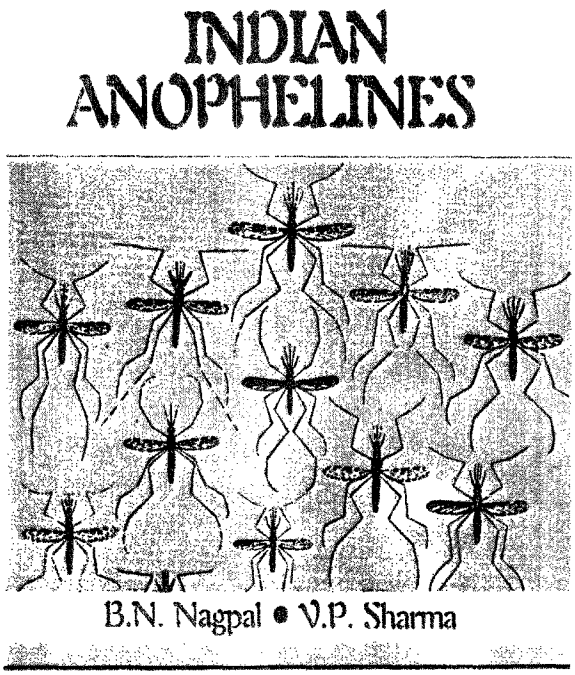

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