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Halofantrine in the Treatment of Falciparum Malaria

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50 patients (45 males + 5 females) suffering from acute uncomplicated attack of *Plasmodium falciparum* (*Pf*) malaria were treated with 1500 mg of halofantrine divided in three doses of 500 mg each given at an interval of 6 h. Results showed there were no primary treatment failures. Average Parasite Clearance Time (av. PCT) was 51.12 h and average Fever Clearance Time (av. FCT) was 31.25 h. Adverse Drug Reactions (ADR) were mild and self limiting. We conclude that halofantrine is a quite safe and effective new antimalarial agent in the treatment of *Pf* malaria cases.

Keywords: Adverse drug reactions, Chloroquine resistance, Halofantrine *Plasmodium falciparum*

INTRODUCTION

According to recent reports from WHO, over 40% of the world population is exposed to the risk of malaria and about 300 million are infected with the malaria parasite with a global death rate of over 3.5 million/year[1]. Resistance of the malaria parasite to antimalarial drugs which was first noted three decades back has increased to an alarming proportions in the last decade and that in turn has encouraged search of new antimalarial agents. Halofantrine (a phenanthrene methanol derivative) is one such synthetic antimalarial agent. Large number of clinical trials have confirmed its

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efficacy and safety in the treatment of *Pf* and *Pv* malaria cases[2-6]. To evaluate the efficacy and safety of halofantrine in Indian patients, a clinical trial was undertaken among the patients suffering from acute *Pf* malaria at Medical College and S.S.G. Hospital, Baroda from September to December 1991.

MATERIALS AND METHODS

After obtaining a prior permission from the Local Ethics Committee, patients showing clinical features of uncomplicated malaria and having a parasitic count of more than 3000/cmm were included in the study. Patients with severe and complicated malaria (as specified by WHO) with significant concomitant multisystem disease; where oral therapy was not possible, patients with G6PD deficiency and pregnant and lactating females were excluded.

Informed and written consent was obtained from all the patients before inclusion in the trial. Following investigations were done in all the patients before and on Day 4 of the treatment: Hb, RBC count, total and differential WBC count, serum bilirubin (SBil), SGOT, SGPT, alkaline phosphatase (ALP), serum electrolytes and blood urea (SEBU), serum creatinine (SCr), urine albumin, sugar and microscopy, urobilinogen, bile salts and pigments.

Malaria parasite count was done from thick smear before starting therapy, at the end of 24 h after administration of halofantrine and subsequently every 12 h till patient tested negative and persisted so for 48 h. It was then repeated on Day 7 in all the patients. Parasitic count was done by calculating the number of parasites/100 WBCs multiplied by total WBC count. Peripheral smear (PS) was labelled negative only on failure to detect malaria parasite after screening minimum twenty fields. Halofantrine was given in 3 doses of 500 mg each at an interval of 6 h. Other symptomatic therapy such as antipyretic or antiemetics were given S.O.S. to all the patients. Patients were examined daily for a period of 7 days for any clinical improvement and development of ADR.

RESULTS

Present study included 50 patients (M/F : 45/5) in the age group of 13-60 yrs, showing symptoms for the duration of 1 to 7 days. It included 45 fresh cases and 5 resistant to chloroquine (CHQ). There were 25 patients (50%) with baseline malaria parasite count between 3000-10,000/cmm, 20 with 10,001-50,000/cmm, 4 with a count of 50,001-100,000/cmm and 1 with more than 100,000/cmm. The average malaria parasite count was 19,780/cmm with a range of 3312-1,48,000/cmm.

Evaluation of drug efficacy: Average PCT was 31.25 h, with a range of 24

to 72 h. Out of 50 patients, 18 became afebrile within 24 h; additional 25 patients became afebrile within 36 h. Fever continued beyond 48 h only in 3 patients. The only patient, who continued to have fever beyond 72 h even after achieving negative PS was suffering from pneumonia and responded to appropriate therapy. Other symptoms such as headache, bodyache, nausea also showed similar favourable response. Table 1 shows effect of halofantrine on haematologic and biochemical parameters. It shows that halofantrine did not alter any of the said parameters adversely.

Table 2 shows effect of halofantrine on parasite count and PCT. Malaria parasite count fell from average of

Table 1. Effects of halofantrine on haematological and biochemical parameters

Parameters	Value on Day 1 (Mean ± 2SD)	Value on Day 4 (Mean ± 2SD)	*z*-values
Hb (gm%)	9.9 ± 2.31	10.4 ± 2.2	
TC/cmm	5000 ± 1637	4438 ± 954	
SGOT (IU/L)	48.46 ± 41.87	38.87 ± 28.32	1.3
SGPT (IU/L)	45.12 ± 44.72	38.23 ± 38.74	0.908
Serum alkaline phosphate (IU/L)	100.16 ± 72.83	89.24 ± 47.6	0.908
Serum bilirubin (mg%)	1.35 ± 0.8	0.87 ± 0.49	3.58*
Blood urea (mg%)	31.77 ± 11.93	25.96 ± 8.59	2.75*
Serum creatinine (mg%)	0.76 ± 0.35	0.57 ± 0.23	2.9*

*$p < 0.05$.

Table 2. Effect of halofantrine on malaria parasite count (MPC) and PCT

Time (h)	Av. MP/ cmm	% fall in MPC	Negative PS in number of patients
0	19,780	-	-
24	3162	84	-
36	-	-	10
48	209	98.95	26
60	-	-	8
72	41	99.80	5
96	0	100	1

19,780/cmm on Day 1 to an average of 3162/cmm at the end of 24 h, thus causing 84% reduction in the count. By the end of 48 h same fell to an average of 209 malaria parasite/cmm thus achieving a drop of 98.95%. By Day 5, cent per cent reduction in malaria parasite count was achieved and average PCT was found to be 51.12 h with a range of 36 to 96 h. Thus there were no primary treatment failures. ADR profile was very mild and self limiting. It included mild vomiting, diarrhoea and abdominal pain in 10, 8 and 6 patients respectively.

DISCUSSION

Malaria parasite by developing drug resistance has found its natural defence against increasing use of antimalarial drugs. The observation of drug resistance to proguanil and pyrimethamine in sixties did not attract much attention since these drugs were not commonly used for treating acute attacks of malaria. However, the resistance to CHQ is of great significance. Initial reports came from Columbia and were later followed by reports from Thailand, Malaysia, Combodia etc. Increased problem of drug resistance necessitated the need for newer antimalarial drugs effective against falciparum resistant to CHQ. Halofantrine seems to fill this vaccum. Various trials carried out with halofantrine have established the safety and efficacy of this drug in acute *Pf* and vivax malaria[3,4,7,8]. The average FCT has varied from 28.50 to 60.30 h in various studies and most of the patients feel significantly better within 24 to 48 h of initiation of therapy. The average FCT in present trial was 31.25 h, thus it was comparable with others. In present series average PCT was 51.12 h with a range of 36 to 96 h, whereas earlier workers reported it to vary between 34 to 78 h.

One of the disadvantage of halofantrine is that the recrudescence rate (RR) in patients treated with it is high and varies between 6 to 30% in various studies. Horton[4] in a large series of 1474 patients reported RR in 6% patients. Exact cause of such variation is unknown but it has been observed that high RR has been associated with lower serum levels of drug (even after administering standard doses), which in turn is thought to be due to unpredictable absorption of the drug in gastrointestinal tract. It is understood that drug administered along with fatty meals increases its absorption probably due to its lipid solubility and is associated with high serum levels and low RR. In present trial we did not study RR because of lack of patient's follow-up beyond 14 days and inability to provide mosquito free atmosphere.

Another disadvantage of the drug is its availability only in oral form, making it unsuitable for treating patients with severe and complicated malaria, where preferred mode of therapy is parentral.

The manufacturers indicate that use of the drug is contraindicated in pregnant females and lactating mothers.

The advantage of the drug is of a very short course of therapy and minimum side-effects resulting in greater patient acceptance and compliance. Regarding the usefulness of the drug in multidrug resistant *Pf* malaria cases, a word of caution is necessary. In some places cross-resistance with mefloquine resistant stains of *Pf* malaria has been reported by some workers. More data is needed on its efficacy against mefloquine resistant strains. A more reliable formulation of halofantrine which could guarantee its better bioavailability is needed.

In conclusion halofantrine is a promising drug. But for its best use, the drug should be given only in multidrug resistant uncomplicated cases of *Pf* malaria.

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Prevalence of Malaria among Pregnant and Non-Pregnant Women of District Jabalpur, Madhya Pradesh

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In the study period of two years 145 pregnant and 79 non-pregnant women with malarial infection were recorded. *Plasmodium falciparum* was the most prevalent species accounting for 72% of the total malaria infection in pregnant women while, in non-pregnant women it accounted for 58%. Results were analysed according to the species to which the parasite belonged, period of gestation and parity. While cerebral malaria, abortions, intrauterine foetal death, maternal anaemia were common in pregnant patients, only one neonatal death was recorded. Malaria parasites were not found in infants born to mothers with very heavy parasitaemia at the time of delivery. Even though pregnant women of all age groups and parity remain highly susceptible to malaria throughout pregnancy and puerperium from this area, some striking differences like malaria infection more prevalent in primigravidas than multigravidas and in second trimester than in third trimester were noticed in comparison to northen India. Results emphasize the need to target malaria control for this group of women. Failure to clear parasitaemia after chloroquine administration in *P. falciparum* was common in both pregnant and non-pregnant women. This is an area, where there is a great need to introduce effective malaria interventions. As chloroquine resistant parasites spread a better understanding of the problem is needed leading to a few chemotherapeutic options for pregnant women.

Keywords: Cerebral malaria, Multigravida, Parity, *Plasmodium falciparum*, Primigravida

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INTRODUCTION

It is generally agreed that during pregnancy women show an increased prevalence of malaria[1], which can cause abortion, intrauterine foetal death, premature delivery and even maternal death[2]. Almost all the published literature on this topic refers to hyperendemic areas of Africa[3], the only work from India is from Chandigarh[4] an area of unstable malaria. McGregor[5] and Hendrickse[6] were of opinion that the course of malaria in pregnancy may be different in areas of stable and unstable malaria. Since no such study had been carried out in Central India (an area of stable malaria), the present study was undertaken to elucidate the prevalence of malaria in pregnant women in Jabalpur and to define some of the effects that it may exert during pregnancy to provide additional information for the development of malaria control strategies.

MATERIALS AND METHODS

Study area: A malaria clinic was established (1991) in the Obstetrics and Gynaecology, Department of Government Medical College, Jabalpur to study the prevalence and pattern of malaria in pregnant and non-pregnant women of Central India (Madhya Pradesh).

Jabalpur is located in the centre of India and has a mixed population of rural, tribal and urban origin. The district has mostly rocky and undulating terrain without proper drainage and irrigation. The Medical College is surrounded from two sides by typical urban slums and people belonging to poor socio-economic strata. Malaria transmission is almost perennial[7] with a definite seasonal trend. Cases in spring (March-June) mainly comprised *Plasmodium vivax* and by the onset of monsoon (July) a peak was observed which later declined to low levels. From August onwards, *P. falciparum* starts increasing, having a peak in November and during mid winter (Dec-Jan) the prevalence of *P. falciparum* was quite high but declined by February. Chloroquine resistance is a common feature[8,9].

Study group: All pregnant women including puerperal (up to 40 days after delivery) attending malaria clinic with a history of fever were screened for malaria parasite by peripheral blood smears. Thick and thin blood films were made from finger pricks and stained with Giemsa[10]. Thin smears were examined under the microscope to determine the species of malaria parasites present. Blood smears were also prepared from infants (up to 40 days). Prevalence of placental infection was not studied in this investigation. For comparison, non-pregnant women of child bearing age with malaria infection during the same period from the same hospital were chosen to serve as control. These women were

recruited from a pool of non-pregnant patients (complaining fever) as they attended the emergency medical outpatient department. The control cases were matched with pregnant women for age, parity and geographical origin. History of fever, clinical findings, parasite species, treatment given and response to treatment were recorded in each case.

Response to chloroquine was studied by a simplified *in vivo* test[11]. Patients were given 1500 mg chloroquine in three dosages (600, 600 and 300 mg). The course of asexual parasitaemia of each patient was evaluated over 2 days. All cases found positive on Day 2 were referred to hospital for quinine therapy. Further drug sensitivity determination was less reliable because patients left hospital very early for financial reasons and did not return for follow-up examinations. The number of asexual parasites per 300 leucocytes was counted and parasite densities were calculated based on a standard value of 8000 leucocytes per microlitre. Serious patients (cases of PET, renal disease and diabetes etc.) and patients with very high parasitaemia (> 20%) were excluded from the study because complications and mortality are influenced by a number of factors which may not be common with normal adults. Percentage parasitaemia was determined from peripheral blood smears by examining 10,000 erythrocytes and calculating the number of parasitized erythrocytes per 100 erythrocytes. An unselected group of 145 pregnant women with malaria were analysed according to parity,

Table 1. Season-wise prevalence of malaria among pregnant and non-pregnant women of district Jabalpur

Season (Months)	Pregnant women				Non-pregnant women			
	BSC	+ve	*Pv*	*Pf*	BSC	+ve	*Pv*	*Pf*
Dry (Mar-Jun)	62	12	10 (83.3)	2 (16.7)	60	7	6 (86.0)	1 (14.0)
Monsoon (Jul-Oct)	466	69	22 (31.9)	47 (68.1)	440	40	23 (57.5)	17 (42.5)
Autumn (Nov-Feb)	303	64	9 (14.0)	55 (86.0)	300	32	4 (12.5)	28 (87.5)
Total	831	145	41 (28.3)	104 (71.7)	800	79	33 (41.7)	46 (58.2)

BSC – Blood slide collection; *Pv* – *P. vivax*; *Pf* – *P. falciparum*; Figures in parentheses indicate percentages.

period of gestation, type of infection and its outcome. Gestational period was calculated as per patient's statement. The normal test (z-test) was used to compare the difference between sample means.

RESULTS

A total of 831 pregnant and 800 non-pregnant women were screened for malaria parasites (Table 1). Out of 145 malaria positive cases among pregnant women, 101 were infected with *Plasmodium falciparum*, 41 with *P. vivax* and 3 had mixed infection with *P. vivax* and *P. falciparum*. These 3 cases were analysed along with the *P. falciparum* cases, as that was the predominant type of infection. Malaria prevalence was significantly higher in pregnant women compared to non-pregnant women ($p<0.01$). Out of 104 cases of *Pf* in pregnant women, 56 (54%) were gametocyte carriers, while in 46 cases of *Pf* in non-pregnant women, 15 (33%) were gametocyte carriers. Number of *P. falciparum* cases and gametocyte carriers were significantly higher in pregnant group compared to non-pregnant group ($p<0.01$). The seasonal distribution of parasite species was similar in both the groups. Table 2 shows that malaria prevalence was greater during the second trimester in both primigravidas and multigravidas.

Table 3 revealed that significantly more pregnant women with malaria were anaemic (Hb < 9 g%) compared to pregnant women without malaria ($p<0.01$). Table 4 shows the impact of malaria on maternal morbidity and mortality as well as perinatal outcome. Out of 104 cases of *P. falciparum*, cerebral malaria occurred in 23 cases of which 16 patients died showing a case fatality rate of 15%. Out of remaining 88 cases of *P. falciparum* in pregnant women, abor-

Table 2. Prevalence of malaria according to gestation period and parity in pregnant women

Gestation period	Mean age (yrs)	Primigravidas/nulliparous			Mean age (yrs)	Multigravidas/multiparous		
		Fever cases	*Pv*	*Pf*		Fever cases	*Pv*	*Pf*
1st trimester	17.00	12	0	1	24.33	18	2	3
2nd trimester	20.81	37	3	13	26.21	60	9	14
3rd trimester	21.04	94	4	21	25.33	217	15	30
Puerperium	21.63	57	2	7	25.50	336	6	15
Total pregnant	20.76	200	9	42	25.68	631	32	62
Non-pregnant	20.04	300	10	16	25.50	500	23	30

Table 3. Classification of cases of anaemia in pregnant women

Gestation period	Pregnant women with malaria Hb (g%)			Pregnant women without malaria Hb (g%)		
	<5	<9	>9	<5	<9	>9
1st trimester	1	3	2	0	4	0
2nd trimester	6	17	16	0	0	36
3rd trimester	2	47	21	3	23	51
Puerperium	8	11	11	6	15	19
Total pregnant	17 (11.7)	78 (53.8)	50 (34.4)	9 (5.7)	42 (26.7)	106 (67.5)

Figures in parentheses indicate percentages.

Table 4. Distribution of malaria cases and its consequences among pregnant and non-pregnant women

Parity	Fever cases	+ve	Outcome of malaria						
			Cerebral malaria	Death	Still birth	Abortions	Neonatal death	Severe anaemia	Moderate anaemia
Primi-gravidas	200	51	14 (27)	9 (17.6)	7 (13.7)	3 (6)*	1 (2)	11 (21)	27 (53)
Multi-gravidas	631	94	9 (10)	7 (7.5)	7 (7.5)+	2 (2)	0 (0)	6 (6.4)	51 (54)
Total	831	145	23 (15.9)	16 (11.0)	14 (9.6)	5 (3.4)	1 (0.7)	17 (11.7)	78 (53.8)
Non-pregnant	800	79	5 (6.3)	2 (2.5)	–	–	– (1.2)	1 (25.3)	20

*Out of three abortions, one occurred in a cerebral malaria patient who later recovered; +Out of seven IUFDs, two occurred in patients of cerebral malaria who survived; Figures in parentheses indicate percentages.

tion occurred in 3 cases, intrauterine foetal death (IUFDs) in 11 cases and neonatal death in only one case. Of the 3 abortions and 11 IUFDs, 1 abortion and 2 IUFDs occurred in the remaining 7 patients of cerebral malaria who later recovered. While in non-pregnant group the case fatality rate in *P. falciparum* was only 2.5%. Table 4 shows that primigravidas had significantly more malaria ($p<0.01$) and more severe complications than multigravidas. Out of 41 cases of *P. vivax* in pregnant patients IUFDs occurred in three (one primigravida and two multigravidas) and abortion in two patients (both primigravidas). All were in third trimester and having high parasitaemia (>2%).

Classification of pregnant and non-pregnant cases according to parasitaemia revealed that 45% of the pregnant patients had heavy parasitaemia (>2%; range 2.1-20, over all mean 6.5%) compared to 10% in the non-pregnant group (range 2.0-8.0, over all mean 2.5%). This difference was statistically significant ($p<0.01$).

Malaria parasites were not recorded within 24 h in eleven neonates born to mothers with very heavy parasitaemia (>5%) at the time of delivery. Of which, one neonate (30 days old) died of *P. falciparum* malaria. No other test could be done due to lack of resources.

The response of *P. falciparum* to chloroquine was studied in 20 and 15 women in pregnant and non-pregnant groups respectively. Of which 5 (25%) pregnant and 3 (20%) non-pregnant remained parasitaemic even after chloroquine intake. These women were administered parenteral quinine and all patients showed parasite within 12 h.

DISCUSSION

More than 40 yrs after launching National Malaria Eradication Programme (NMEP) malaria still continues to be a major public health problem in Central India and thousands of pregnant women are exposed to the risk.

During the study period 145 cases of malaria were detected in 3367 pregnant women (the total number of deliveries registered in the Medical College Hospital during the study period). The incidence of malaria during pregnancy was 43 per 1000 deliveries (4.3%). Malaria was recorded in 5 (2.3%) out of 212 abortions and in 14 (6%) out of 236 still birth. There were 94 total maternal deaths, of which 16 (17%) were due to cerebral malaria. In the present study, we found more cases of cerebral malaria and maternal deaths which is in contradiction to the earlier studies from Chandigarh[4].

Significantly higher number of pregnant patients had malaria parasitaemia for both types of infection as compared to non-pregnant women. This is in agree-

ment with most of the studies reported from Africa and northern India. In this study, we failed to detect parasitaemia in the peripheral blood of the new born babies. The only neonate positive for *P. falciparum* showed no malaria parasite in the initial blood smear collected within 24 h after birth. Whether this baby acquired infection postnatally or a case of congenital malaria is a matter of conjecture. Bruce-Chwatt[1], Blacklock and Gordon[12] and Covell[13] reported that congenital malaria is very rare in endemic immune areas. On the contrary, Kortmann[14] and Reinhardt *et al.*[15] recorded an incidence of 3.8 and 21% in newborns of Tanzania and Ivory coast respectively. Menon[16] also described two cases of congenitally acquired infections in newborn in Malaysia. Much work remains to be done on these aspects of malaria in such malarious areas.

Malarial infection was more prevalent in the second trimester and in primigravidas. The importance of parity and the trimester of pregnancy emphasizes the need to target malaria control strategies to primigravidas especially during the first trimester. But most pregnant women do not generally visit hospital until they are 4 or 5 months into their pregnancy. History of illness revealed that women in this part of India were quite unaware of malaria and often suffer from repeated attacks of malaria. Illness is neglected for a long time before reporting to hospital and they generally avoid taking medicines and take short treatment only. Therefore, a regular screening of pregnant women for malaria is required in such endemic areas to reduce morbidity and mortality due to malaria in both women and children. Chemoprophylaxis using chloroquine in suppressive doses (5 mg/kg body weight weekly) has been recommended to protect pregnant women in malarious areas from the adverse effects of malaria during pregnancy[17]. But in areas of chloroquine resistance, weekly doses of 5 mg/kg body weight would not effectively maintain the peripheral blood free of parasite[18]. The chloroquine resistant *P. falciparum*, is common in and around Jabalpur[19]. In this study also persistance of parasitaemia after an appropriate dose of chloroquine is common in both pregnant and non-pregnant women.

There is an urgent need of extensive population based randomised trial of chloroquine prophylaxis in pregnant women to study whether chemoprophylaxis would reduce morbidity and mortality in such endemic areas or not. An understanding of drug efficacy in the target population is an important prerequisite to develop an effective antimalarial policy for pregnant women.

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Larvicidal Activity of a Few Plant Extracts Against *Culex quinquefasciatus* and *Anopheles stephensi*

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Larvicidal activity of partially purified extracts of leaves of *Vitex negundo*, *Nerium oleander* and seeds of *Syzygium jambolanum* on different instars of *Culex quinquefasciatus* and *Anopheles stephensi* was estimated. Petroleum ether (PE) : Ethyl acetate (EA) 3:1 fraction of *V. negundo*, 1:1 fractions of *N. oleander* and *S. jambolanum* inflicted considerable larval mortality and interfered with pupal-adult metamorphosis. At very low concentration the active fractions of these plant extracts extended the duration of the various larval instars and of pupation. In general, I and II instar larvae were more susceptible to the active fractions. Species and stage specific differences in the susceptibility of the mosquitoes to the active fractions of the plant extracts were observed.

Keywords: *Anopheles*, *Culex*, Mosquito control, Plant products

INTRODUCTION

Plants have evolved a variety of secondary compounds; some of them for providing protection from phytophagous insects. Owing to the fact that application of synthetic insecticides results in undesirable consequences on the environment and on the non-target organisms, plant extracts with insecticidal property are used in indigenous methods of control of pest insects and vectors. Following the demonstration of insect growth regulatory effects of

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azadirachtin[1,2] extensive research is being made to explore other plant products with insecticidal property. Regunatharao *et al.*[3] have demonstrated larvicidal activity of azadirachtin– enriched neem preparations on IV instar *An. culicifacies* (Giles) and *Cx. quinquefasciatus* (Say) larvae. Zebitz[4,5] studied the effect of neem kernel extract on several species of mosquitoes. Larvicidal activity of partially purified acetone extract of *Melia volkensi* and *Melia azediracht* against *Cx. pipiens molestus* and *An. arabiensis* has been demonstrated by Mwangi and Mukiama[6] and Al-Sharook *et al*[7]. The larvicidal activity of extracts from three different plants against *Cx. quinquefasciatus* and *An. stephensi* are reported in this paper.

MATERIALS AND METHODS

Mature leaves of *Vitex negundo* L. (F. Verbenaceae), *Nerium oleander* L. (F. Apocynaceae) and seeds of *Syzygium jambolanum* (Lam.) DC. (F. Myrtaceae) were collected and dried in the laboratory under shade. The dried materials were powdered in a kitchen machine and extracted thrice in analytical grade methanol (MeOH) in the ratio of 1:10 w/v. The MeOH extract was evaporated to dryness in a rotary evaporator at 45^{0}C under low pressure. The residue was defatted by washing it with equal volume of MeOH and petroleum ether (PE). Preliminary tests revealed that the defatted MeOH fraction showed considerable larvicidal activity. Hence, this fraction was concentrated and fractionated in a silica gel column using petroleum ether : ethyl acetate (EA) in the ratio of 3:1, 1:1, 100% and 100% MeOH as mobile phase and silica gel (60-120 mesh) as stationary phase. The different fractions were collected and tested against different instars of *Cx. quinquefasciatus* and *An. stephensi*. The strains of mosquitoes were obtained from the ICMR Regional Centre in Madurai and colonized in our laboratory. Eggs were hatched in plastic troughs (25 cm dia) and the larvae were provided with powdered yeast and dog biscuit in the ratio of 3:1. After the completion of the larval development the pupae were transferred to smaller troughs (10 cm dia) and allowed to emerge in standard cages (35x35x35 cm). The freshly emerged females were allowed to feed on an immobilized fowl and the males were provided with 10% sucrose solution. Static bioassay tests was performed in 200 ml glass bowls. To 100 ml of 0.08% saline water[7] required volume of 1% stock solution of the different fractions in MeOH was added and 25 freshly moulted larvae of *Cx. quinquefasciatus* or *An. stephensi* were released into the bowls. Two different controls were maintained. In one of the controls the larvae were exposed to 100 ml (0.08%) saline water alone, while in other larvae were exposed to 100 ml saline water containing appropriate volume of MeOH. The volume of MeOH added correspondes to the volume of stock solution added to obtain the highest tested concen-

Table 1. Twenty four hour LC50, and 95% fiducial limits of the active fractions of the tested plant extracts

Material/ Fraction	Instar	LC50 (ppm)	Fiducial limits	χ^2	SE
Cx. quinquefasciatus					
V. negundo	I	20.09	1.66–1.53	1.69	0.849
PE:EA (3:1)	II	8.23	0.70–0.64	0.81	0.862
	III	42.43	141.07–32.60	2.56	1.559
	IV	35.11	11.53–8.68	1.69	O.303
An. stephensi					
V. negundo	I	36.87	30.59–16.72	2.56	1.72
PE:EA (3:1)	II	36.71	6.84–5.77	2.89	0.47
	III	135.50	39.33–30.49	4.41	1.82
	IV	91.10	35.46–25.53	3.61	1.55
Cx. quinquefasciatus					
N. oleander	I	43.85	13.06–10.06	2.89	0.454
PE:EA (1:1)	II	23.37	2.84–2.54	1.96	0.508
	III	20.20	2.77–2.43	1.96	0.426
	IV	76.40	315.25–61.50	4.00	0.497
An. stephensi					
N. oleander	I	19.82	13.03–7.86	1.69	1.41
PE:EA (1:1)	II	14.85	0.95–0.90	1.21	1.07
	III	72.16	34.30–23.20	3.61	1.83
	IV	37.03	48.80–21.06	2.89	0.94
Cx. quinquefasciatus					
S. jambolanum	I	78.62	16.07–13.34	2.89	0.49
PE:EA (1:1)	II	43.87	12.97–10.11	2.89	1.79
	III	194.34	5.35–5.20	4.84	2.74
	IV	228.68	19.08–17.62	5.29	1.57
An. stephensi					
S. jambolanum	I	81.53	8.83–7.97	3.24	1.004
PE:EA (1:1)	II	84.61	8.04–7.35	3.24	0.725
	III	247.07	30.05–26.79	4.84	0.742
	IV	175.37	21.25–18.95	4.84	0.684

tration. The level of the test solution in the bowls was maintained every day by adding required volume of distilled water. Yeast powder and dog biscuit powder in the ratio of 3:1 were provided as nutrients for the larvae. Mortality of the larvae was monitored continuously and noted. Observations of the treated larvae were continued and the duration required for successive moults and number of adults emerged were noted. Separate bioassay tests were conducted for the different instars. Two replicates consisting of 25 larvae each were maintained for each tested concentration. Using a probit programme based on the suggestions of Finney[8] LC50 of the different fractions for the different instars were computed in a personal computer.

RESULTS

Observation on the mortality of the larvae as a function of instar and concentration of the different fractions of *V. negundo*, *N. oleander* leaf extract and *S. jambolanum* seed extract points out that irrespective of the mosquito species, PE:EA (3:1) fraction of *V. negundo* leaf extract and PE:EA (1:1) fractions of *N. oleander* leaf extract and *S. jambolanum* seed extract have significant larvicidal activity. For instance, 80 ppm of PE:EA (3:1) fraction of *V. negundo* leaf extract 300 ppm of PE:EA (1:1) fractions of *N. oleander* leaf extract and *S. jambolanum* seed extract inflicted about 100% mortality of *Cx. quinquefasciatus* larvae. Table 1 provides 24 h LC50 of the active fractions of the tested plant materials. A comparison of the LC50 of the active fractions for *Cx. quinquefasciatus* points out that *V. negundo* PE:EA (3:1) fraction is more toxic than PE:EA (1:1) fractions of *N. indicum* and *S. jambolanum*. On the other hand LC50 of PE:EA (1:1) fraction of *N. oleander* for *An. stephensi* is less than that of the active fractions of the other two plants (Table 1). In general, the second instar larvae were more susceptible to the active fractions than the other instars.

Tables 2-4 provide data on the duration of the different instars and survival of larvae of *Cx. quinquefasciatus* treated with the active fractions. A careful analysis of the data reveals that the durations of the different instars of the treated larvae were significantly ($p < 0.05$) longer than the corresponding durations of the larvae in the control. However, between the different concentrations the duration did not significantly ($p > 0.05$) vary. Therefore, it may be concluded that the presence of one or the other of the active fractions in the medium even at lower concentrations extends the duration of the different instars. For the larvae treated with the active fractions during the I instar, the effect on duration of the different instars continued till the larvae entered the pupal stage and emerged. Another interesting observation was that the active fractions seriously impaired pupal-adult metamor-

Table 2. Instar duration ($\bar{X}$±SD) of *Cx. quinquefasciatus* larvae treated with PE:EA (3:1) fraction of *V. negundo* leaf extract at the commencement of I, II, III or IV instar

Conc. (ppm)	Instar				Pupa	Survival %
	I	II	III	IV		
Control (MeOH)	2.08±0.28	2.14±0.35	2.15±0.36	2.11±0.31	1.21±0.41	92
5	3.73±1.08	3.77±0.71	3.78±0.73	3.93±0.85	1.72±0.72	88
10	3.81±0.76	3.68±0.73	4.20±0.89	4.04±0.74	1.52±0.55	84
20	4.04±0.89	3.57±0.51	3.30±0.97	3.30±0.47	1.41±0.51	
24						
40	3.50±0.57	4.00±0.00	4.00±0.00	4.00±0.00	1.50±0.70	4
Control (MeOH)	–	2.12±0.33	2.06±0.25	2.08±0.28	1.08±0.28	90
5	–	3.55±0.55	3.34±0.62	3.58±0.50	1.50±0.52	24
10	–	3.23±0.43	3.18±0.40	4.30±0.48	1.28±0.48	14
20	–	4.00±0.00	4.00±0.00	4.00±0.00	1.50±0.00	0
40	–	4.00±0.00	4.00±0.00	4.00±0.00	–	0
Control (MeOH)	–	–	2.08±0.27	2.02±0.14	1.02±0.14	90
5	–	–	4.12±0.74	3.90±0.72	1.37±0.57	48
10	–	–	3.53±0.60	4.03±0.74	1.44±0.50	50
20	–	–	4.36±0.48	3.84±0.68	1.30±0.48	20
40	–	–	4.01±0.72	3.28±0.48	1.40±0.54	10
Control (MeOH)	–	–	–	2.08±0.27	1.04±0.20	96
5	–	–	–	3.65±0.72	1.54±0.61	75
10	–	–	–	3.97±0.86	1.54±0.00	62
20	–	–	–	3.32±0.47	1.15±0.36	40
40	–	–	–	3.50±0.81	1.37±0.51	16
60	–	–	–	3.41±0.50	1.00±0.00	8

Note: The experiment was started with two replicates of 25 larvae each and the observations were continued till adult emergence.

phosis of the survivors even at lower concentrations. For instance, treatment of *Cx. quinquefasciatus* with 20 ppm of PE:EA (3:1) *V. negundo* fraction resulted in the emergence of 24% of the treated larvae into adults (Table 2). Similarly, treatment of the I instar with 20 ppm of PE:EA (1:1) fraction of *N. oleander* extract resulted in the emergence of only 20% of the larvae (Table 3). Most of the pupae could not tear-off the pupal-case especially

in the thoracic and abdominal regions; the head alone could come out of the pupal-case and the thorax was swollen (Fig. 1). Similar instances of failure of emergence of surviving pupae into normal adults was also observed in treatments with different concentrations of PE:EA (1:1) fraction of *S. jambolanum* extract (Table 4).

DISCUSSION

A variety of plants are reported to have insecticidal property[9]. Among the plants selected for the present study,

Table 3. Instar duration ($\bar{X}$±SD) of *Cx. quinquefasciatus* larvae treated with PE:EA (1:1) fraction of *N. oleander* leaf extract at the commencement of I, II, III or IV instar

Conc. (ppm)	Instar				Pupa	Survival %
	I	II	III	IV		
Control (MeOH)	2.04±0.19	2.06±0.24	2.08±0.27	2.02±0.14	1.06±0.24	94
20	4.96±0.73	4.30±0.48	4.30±0.49	4.30±0.47	1.20±0.42	20
40	4.40±0.64	4.50±0.51	4.50±0.51	4.40±0.50	1.40±0.51	20
60	4.10±0.38	4.30±0.50	4.50±0.52	4.25±0.46	1.30±0.51	12
80	4.29±0.46	4.2±0.42	4.50±0.53	4.57±0.53	1.25±0.50	8
Control (MeOH)	–	2.14±0.35	2.02±0.14	2.00±0.00	1.00±0.00	94
10	–	4.72±0.75	4.64±0.67	4.51±0.62	1.46±0.50	56
20	–	4.50±0.57	4.50±0.58	4.47±0.51	1.50±0.51	28
40	–	4.42±0.53	4.60±0.54	4.66±0.57	–	0
60	–	4.50±0.70	–	–	–	0
Control (MeOH)	–	–	2.08±0.27	2.10±0.30	1.10±0.37	96
10	–	–	4.67±0.71	4.62±0.60	1.60±0.62	56
20	–	–	4.38±0.57	4.28±0.46	1.30±0.50	18
40	–	–	4.26±0.45	4.50±0.52	1.30±0.57	6
60	–	–	4.30±0.57	5.00±0.00	–	0
Control (MeOH)	–	–	–	2.06±0.23	1.06±0.25	88
10	–	–	–	4.87±0.89	1.51±0.50	66
20	–	–	–	4.50±0.60	1.50±0.63	56
40	–	–	–	4.69±0.48	1.30±0.50	18
60	–	–	–	4.60±0.57	–	0

Note: The experiment was started with two replicates of 25 larvae each and the observations were continued till adult emergence.

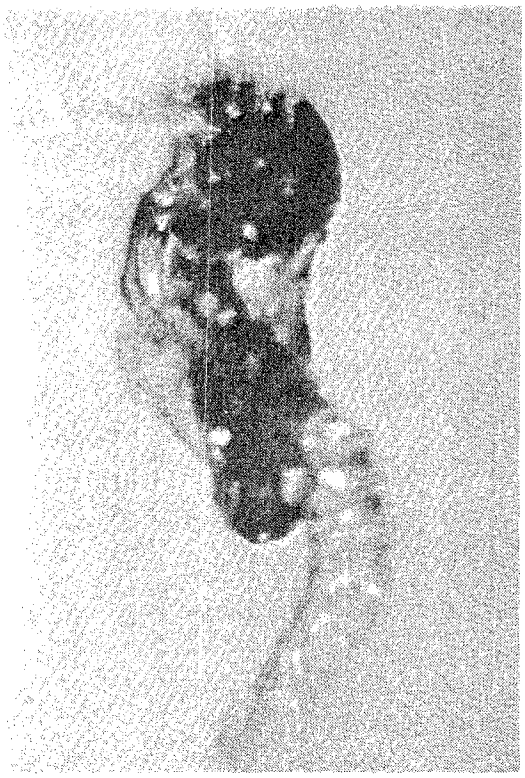

Fig 1: Pupal-adult intermediate of *Culex quinquefasciatus*

V. negundo has been reported to have antifeedant, repellent and toxic properties against insect pests[10,11]. Saravanan[12] demonstrated that oral administration of seed extract of *S. jambolanum* significantly decreased the blood sugar level of mice. Therefore, it is likely to influence the synthesis of chitin— an important compound required for the synthesis of cuticle in insects. Different parts of *N. oleander* are traditionally known to have toxic effects on mammals. The present study has provided ample evidences for the existence of compounds with insect growth regulatory activity in the active fractions of these plant extracts. Kernel extracts of *Azadirachta indica*[5], *Melia volkensi*[13], *Melia azediracht*[7] and whole plant extract of *Lithospermum arvense*[14] have been shown to have significant larvicidal activity against different species of mosquitoes. However, these investigators have chosen a particular instar of the mosquitoes and hence could not compare the vulnerability of the different instars to the tested plant materials. In the present study, the active fractions have been tested against all the larval stages of *Cx. quinquefasciatus* and *An. stephensi*. The results have shown that the fractions were more toxic to the II or I instar larvae. Al-Sharook *et al.*[7] reported LD50 of 20-30 ppm and 30-40 ppm of acetone extracts of *M. volkensi* and *M. azediracht* kernels to III instar *Cx. quinquefasciatus* larvae. Pure azadirachtin was many fold more active (LD50=1-5 ppm) than the acetone extracts of *M. volkensi* and *M. azediracht*. In the present study, LC50 of PE:EA (3:1) *V. negundo* fraction ranged between 8.2 to 42.4 ppm for the different instars of *Cx. quinquefasciatus* and 36.7 and 135.5 ppm for the different instars of *An. stephensi*. Therefore, the larvicidal activity of *V. negundo* fraction is on par with that of *M. azediracht* kernel extract reported by Al-Sharook *et al.*[7] Comparison of the LC50 of the different active fractions suggests that the larvicidal activity of the fractions varies with the species as well as with the

instar. Al-Sharook *et al.*[7] have also reported stage specific differences in the activity of *M. volkensi* and *M. azediracht* kernel extract against *Cx. quinquefasciatus*. Mwangi and Rembold[13] have shown differential activity of azadirachtin-A on *Cx. quinquefasciatus* and *Ae. aegypti*. Similarly, Zebitz[5] reported that the activity of the neem kernel extract, azadirachtin and altosid (a JH analogue) varied between different species of *Culex*, *Aedes* and *Anopheles*. It is perhaps, the differences in the chronological events tak-

Table 4. Instar duration ($\bar{X}$±SD) of *Cx. quinquefasciatus* larvae treated with PE:EA (1:1) fraction of *S. jambolanum* seed extract at the commencement of I, II, III or IV instar

Conc. (ppm)	Instar treated				Pupa	Survival %
	I	II	III	IV		
Control (MeOH)	2.04±0.19	2.04±0.20	2.06±0.24	2.00±0.00	1.00±0.00	90
20	4.17±0.38	4.29±0.46	4.38±0.49	4.50±0.56	1.51±0.50	62
60	4.36±0.49	4.92±0.07	4.43±0.50	4.36±0.49	1.42±0.50	42
100	4.25±0.44	4.31±0.47	4.50±0.51	4.41±0.51	1.37±0.51	16
160	4.23±0.43	4.37±0.51	4.50±0.54	4.40±0.54	1.33±0.57	6
200	4.37±0.51	4.75±0.50	4.50±0.70	4.0±0.00	1.50±0.00	2
Control (MeOH)	–	2.04±0.19	2.04±0.20	2.02±0.14	1.02±0.14	92
20	–	4.50±0.65	5.00±0.74	4.52±0.50	1.50±0.51	40
60	–	4.48±0.50	4.42±0.05	4.40±0.50	1.28±0.48	14
100	–	4.78±0.71	4.40±0.51	4.60±0.50	1.66±0.57	6
160	–	4.30±0.57	4.50±0.70	4.00±0.00	1.50±0.00	2
Control (MeOH)	–	–	2.04±0.19	2.06±0.23	1.08±0.28	90
20	–	–	4.40±0.64	4.59±0.72	1.60±0.67	80
100	–	–	4.53±0.63	4.58±0.66	1.57±0.69	70
200	–	–	4.48±0.77	4.50±0.76	1.50±0.73	32
300	–	–	4.75±0.50	4.66±0.57	1.50±0.70	4
Control (MeOH)	–	–	–	2.04±0.19	1.06±0.24	94
20	–	–	–	4.63±0.67	1.60±0.66	82
100	–	–	–	4.51±0.63	1.60±0.69	70
200	–	–	–	4.46±0.57	1.60±0.68	40
300	–	–	–	4.21±0.42	1.30±0.50	18

Note: The experiment was started with two replicates of 25 larvae each and the observations were continued till adult emergence.

ing place during the larval development of the different species which is responsible for the differential susceptibility of the different species to the plant products. The findings that the active fractions obtained in the present study extended the duration of the different instars and interfered with moulting and pupal-adult metamorphosis points out that the fractions act through the endocrine system of the larvae. Clearly, the active fractions effectively inhibit the growth of the larvae and prevent pupal-adult metamorphosis and hence, have a promising future in the control of mosquitoes.

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Indoor Resting Anophelines of North Bengal

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A systematic survey was carried out between April 1993 and March 1994 to study the distribution and prevalence of anopheline mosquito species in two malaria-prone areas situated in the foothills of Darjeeling district. Ten different species of *Anopheles* viz. *An. aconitus*, *An. annularis*, *An. barbirostris*, *An. culicifacies*, *An. fluviatilis*, *An. hyrcanus* group, *An. maculatus*, *An. subpictus*, *An. vagus* and *An. varuna* were collected. Per man hour density of mosquitoes collected was 4.5 and the most prevalent species was *An. vagus*, comprising 63% of the total catch. *An. fluviatilis*, an efficient vector of malaria in the foothill regions of India, was reported for the first time from this area. However, the classical vector of this region viz. *An. minimus* was altogether absent during the course of the present survey. All the ten species were found in cowsheds while, human habitation harboured higher population density (56.5%) of the total catch.

Keywords: *Anopheles*, Foothill, Himalayas, Seasonal prevalence

INTRODUCTION

The resurgence of malaria in the recent past in different areas adjacent to Darjeeling hills has aroused considerable interest to study the anopheline fauna of this region. Drastic changes in ecological conditions owing to extensive and illegal deforestation, huge automobile exhaust, rapid and unplanned urbanisation has led to an alteration in species composition and

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their relative abundance in this unique habitat. Information on the anopheline fauna of the foothill regions of Darjeeling district, particularly the species involved in local malaria transmission, is still inadequate. Earlier, Rao *et al.*[1], Bhat[2] and Rao[3] conducted sample survey in different areas of Darjeeling district. No systematic and comprehensive survey has been made so far in the recent past. However, Malakar *et al.*[4] has added a preliminary note on the anopheline fauna of some areas of Darjeeling district during winter season.

MATERIALS AND METHODS

The present study was conducted between April 1993 and March 1994. Two malaria-prone areas namely Sukna (Altitude 169.5 m) and Rongtong (Altitude 482 m) situated in the foothills of Darjeeling Himalayas were selected for this purpose. The study area was characterised by thick forest, heavy rainfall, slow running hill streams, forest bustees; with more than 60% of inhabitants from low socio-economic communities.

In the Rongtong area, indoor collections were made from three selected cowsheds and three human habitations (mud-house) during morning hours (0600 to 0700 hrs). In the Sukna area, two malaria-prone villages namely Jonglikotha and Khairani-Punding were selected for the present study and three fixed cowsheds and human habitations were searched thoroughly for adult collections. Each study area was visited twice a month spending 4 man hours/month/study area (2 man hours in human habitations and 2 man hours in cowsheds).

Mosquitoes were collected manually with the help of test tubes and battery–operated torch. The specimens were identified following the keys of Roy and Brown[5], labelled and preserved. Indoor temperature and humidity were also recorded during each collection. The density per man hour (MHD) for each species was calculated according to the time spent and nature of habitat.

RESULTS AND DISCUSSION

During the study period a total of 646 adult female anopheline mosquitoes representing ten different species viz. *An. aconitus*, *An. annularis*, *An. barbirostris*, *An. culicifacies*, *An. fluviatilis*, *An. hyrcanus* group, *An. maculatus*, *An. subpictus*, *An. vagus* and *An. varuna* were collected from three different areas in the foothills after spending a total of 144 man hours. The order of species dominance found was *An. vagus* (63%), *An. culicifacies* (11.45%) and *An. subpictus* (10.8%). In the Rongtong area (altitude 480 m), *An. maculatus* was recorded to be the second dominant species with a population density of

Table 1. Monthwise occurrence of different species of *Anopheles* in Rongtong area (April 1993-March 1994)

Species	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Total	MHD
An. aconitus	1	–	–	–	–	1	–	–	–	–	–	–	2	0.04
An. annularis	–	2	–	–	–	–	–	–	–	–	2	–	4	0.08
An. culicifacies	11	5	–	2	–	–	–	–	–	–	2	1	21	0.44
An. fluviatilis	–	–	–	–	–	–	–	–	–	1	–	–	1	0.02
An. hyrcanus group	–	–	–	–	–	–	–	–	–	–	1	–	1	0.02
An. maculatus	6	2	–	2	2	2	6	1	4	–	2	5	32	0.67
An. subpictus	7	1	1	–	1	–	–	–	–	–	–	–	10	0.21
An. vagus	27	18	50	2	13	11	–	–	–	–	–	2	123	2.56
An. varuna	1	–	–	–	–	–	–	–	–	–	–	–	1	0.02
Total	53	28	51	6	16	14	6	1	4	1	7	8	195	4.06
Av. temperature (°C)	24.7	27	26.2	26.4	25.6	25.3	22.3	19.8	18.3	18.3	13.6	22		
Relative humidity (%)	56.5	58	62	65	70.3	68	58	60.7	54	53.8	57	52.7		

Table 2. Monthwise occurrence of different species of *Anopheles* in Sukna area (April 1993-March 1994)

Species	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Total	MHD
An. aconitus	–	–	1	–	–	–	–	–	–	–	1	1	2	0.02
An. annularis	1	4	1	3	3	–	2	–	–	1	2	–	17	0.18
An. barbirostris	–	–	–	–	–	1	18	2	–	1	–	–	22	0.23
An. culicifacies	20	25	4	1	1	2	–	–	–	–	–	–	53	0.55
An. hyrcanus group	–	–	–	–	–	–	–	–	–	–	1	–	1	0.01
An. maculatus	–	–	–	–	–	2	–	2	–	–	7	-	11	0.11
An. subpictus	41	18	–	–	1	–	–	–	–	–	–	–	60	0.62
An. vagus	7	90	42	19	44	18	8	2	–	–	–	55	285	2.97
Total	69	137	48	23	49	23	28	6	–	2	11	56	451	4.69
Av. temperature (°C)	27.7	27	28.7	28.7	27	26.9	25	20	17.85	14.5	19.4	24.5		
Relative humidity (%)	56.8	53.6	55.4	57.5	63.8	62	58.6	62	58.5	58.3	56.3	54.3		

0.66 MHD while at a lower altitude at Sukna (169.5 m), *An. subpictus* occupied the second position (0.62 MHD); (Tables 1 and 2).

An. bengalensis, *An. lindesayi* and *An. interruptus* reported earlier by Rao *et al.*[1], Bhat[2] and Rao[3] from the foothill regions of Darjeeling including Sukna were found absent. It is also to be noted that the earlier proven vector of this region viz. *An. minimus*[6,7] was also totally absent during the course of the present study. This finding lends support to the earlier findings of Varma and Mahadevan[8] and Rao[3], who didn't record the species from this area. However, Bhat[2] and Hati[9] collected this species, though numerically negligible from this area. The near disappearance of the classical vector may be attributed to the previous NMEP activities and abrupt change in ecological conditions like extensive deforestation as suggested by Sharma *et al.*[10] in the case of *An. fluviatilis* in Uttar Pradesh.

Interestingly, a single specimen of *An. fluviatilis* was isolated from the cowsheds in the Rongtong area in January and was reported for the first time from the hilly terrains of Darjeeling Himalayas. The species is characteristically a hill or foothill species and based on several evidences. Pradhan *et al.*[11] opined that it is one of the most efficient vectors of malaria in India. Particularly in the hills and foothills, it is extraordinarily efficient, perhaps more efficient than any other Indian *Anopheles*.

An. barbirostris was completely absent in Rongtong collection and was collected from Sukna only during post-monsoon period and disappeared altogether from February to September. The species has also been recorded earlier from Darjeeling district by Rao *et al.*[1]

A single specimen of *An. varuna* was isolated from the cowsheds of Rongtong area which confirm the earlier report of Bhat[2].

Of the two different biotopes examined viz. human habitation and cowshed, the former harbours higher population density (56.5%) than the latter. Cowsheds were found to contain the richest anopheline fauna and all the ten species have been isolated from this habitat. The lower density of anopheline species in the cowsheds may be attributed to the fact that most of the cowsheds in the study area were of open type (Table 3).

Among individual species *An. annularis* (86%) and *An. maculatus* (93%) showed marked preference toward cattlesheds, whereas the adults of *An. barbirostris* and *An. vagus* were more prevalent in human habitations. However, Kulkarni[12] reported the occurrence of

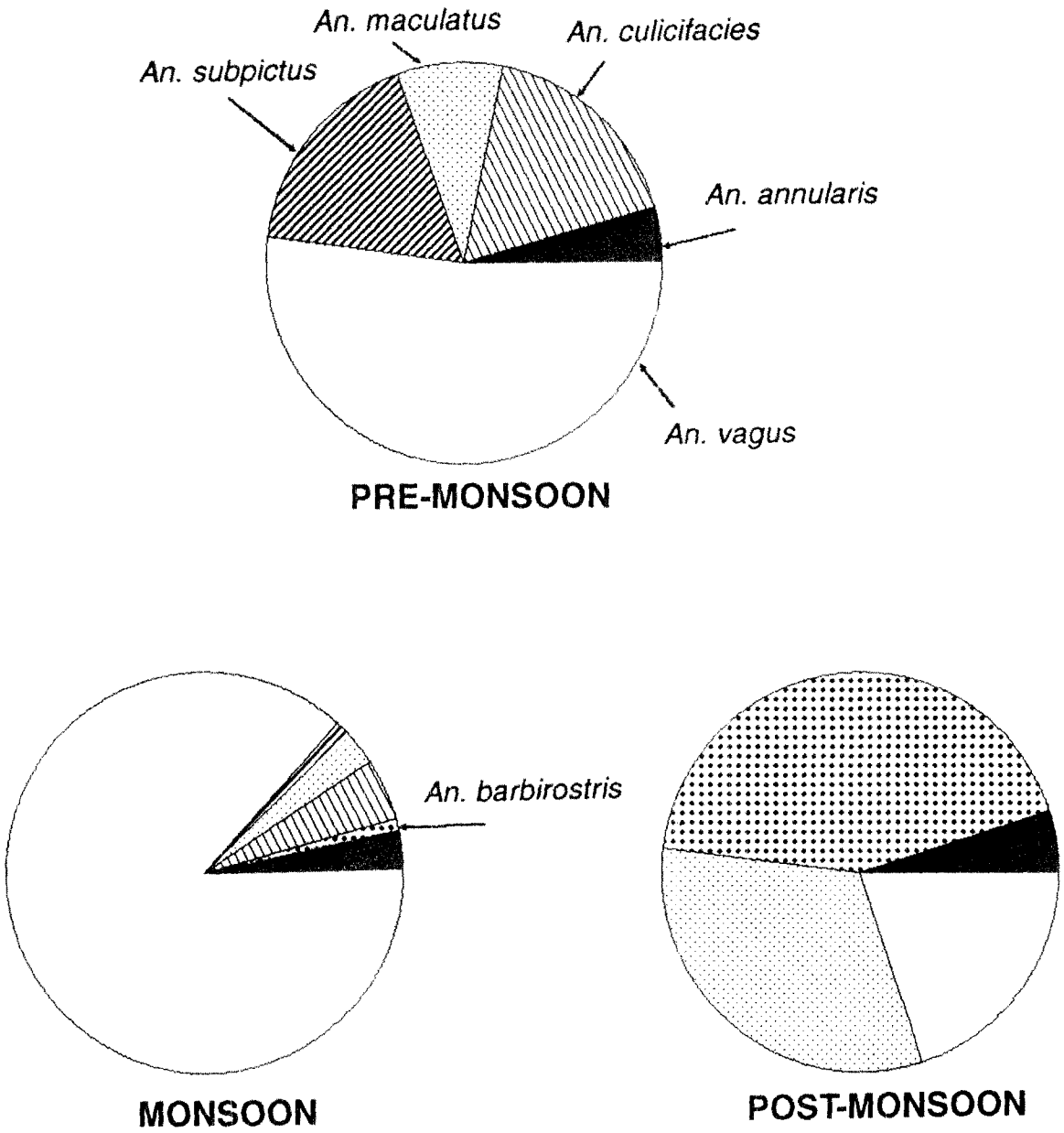

Fig. 1: Trend of seasonal fluctuations of some common species of *Anopheles* in the foothills of Darjeeling Himalayas

An. barbirostris adults universally in cattle and buffalosheds. *An. subpictus* and *An. culicifacies* were present both in houses and cowsheds (Table 3).

The trend of seasonal prevalence of six common species of *Anopheles* showed that in most of the cases, the peak population density occurred during pre-monsoon season as shown in Fig. 1.

It was observed that *An. culicifacies* was abundant during pre-monsoon; the period from June to September was also found favourable but the species disappeared completely from October to January, indicating their pre-monsoon breeding habit (Table 4).

An. maculatus showed two peaks, the highest being 51% during pre-monsoon season followed by 34.8% in the post-monsoon season (Table 4). Since they usually breed in stream, there are chances being washed away due to heavy rainfall during the monsoon. The species was reported to prefer outdoor resting by earlier workers[2,13]. However, all the specimens during the present survey were collected from indoor habitat.

A fairly good number of *An. subpictus* were collected from both the study areas throughout the year with a peak density during pre-monsoon period. Earlier, Strickland *et al.*[14] also reported the species from the terai region of Darjeeling with two sporozoite positivity. However, Varma and Mahadevan[8] in their collection could not find any specimen from the Sikkim and West Bengal areas of the Himalayas.

Table 3. Habitat preference of different species of *Anopheles* in the foothills of Darjeeling Himalayas

Species	Human habitation	Cowshed	Total
An. aconitus	2 (50)	2 (50)	4
An. annularis	3 (14)	18 (86)	21
An. barbirostris	16 (72.7)	6 (27.2)	22
An. culicifacies	42 (56.8)	32 (43.2)	74
An. fluviatilis	–	1 (100)	1
An. hyrcanus group	1 (50)	1 (50)	2
An. maculatus	3 (6.9)	40 (93.1)	43
An. subpictus	39 (56)	31 (44)	70
An. vagus	259 (63.4)	149 (36.6)	408
An. varuna	–	1 (100)	1
Total	365 (56.5)	281 (43.5)	646

Figures in parentheses are in percent.

Table 4. Trend of seasonal variations of common species of *Anopheles* in the foothills of Darjeeling Himalayas

Species	Pre-monsoon (Feb-May)	Monsoon (Jun-Sep)	Post-monsoon (Oct-Jan)	Total
An. annularis	11 (52.3)	7 (33.3)	3 (14.2)	21
An. barbirostris	–	1 (4.5)	21 (95.4)	22
An. culicifacies	64 (86.4)	10 (13.5)	–	74
An. maculatus	22 (51.1)	6 (13.9)	15 (34.8)	43
An. subpictus	67 (95.7)	3 (4.3)	–	70
An. vagus	199 (48.7)	199 (48.7)	10 (2.4)	408
Total	363 (57)	226 (35.4)	49 (7.7)	638

Figures in parentheses are in per cent.

The population density of *An. annularis*, a malaria-vector of secondary importance[15] and *An. vagus* the most prevalent species of the foothills was higher in the pre-monsoon and monsoon seasons and both the species disappeared gradually during post-monsoon season showing more or less perennial breeding.

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Naturally Acquired Concomitant Infections of Bancroftian Filariasis and Human Plasmodia in Orissa

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Blood smears collected from fever cases for detection of malaria parasites during daytime showed concomitant infections of *Wuchereria bancrofti* from 1989 to 1991 in Bisra PHC of district Sundargarh, Orissa. Of the total 51,448 blood smears examined, 18,444 (35.84%) were positive for malaria parasites which comprised 3401 (18.44%) *Plasmodium vivax*, 14,524 (78.75%) *P. falciparum*, 156 (0.84%) *P. malariae* and 363 (1.97%) mixed plasmodial infections. Only 240 (0.46%) cases were positive for *W. bancrofti*, of which 160 (66.67%) were frank microfilariae (mf) cases, while 80 (33.33%) showed concomitant infections with malaria parasites. Filariasis was less prevalent in lower age-groups. Malaria incidence in people below thirty years was higher compared to older people, on the contrary, mf incidence was more in people above 15 yrs or more age. Microfilariae density was within 1-7 parasites per 10 µl blood. About 90% mf cases were within the range of 1-4 per 10 µl blood. Mean malaria parasitaemia in concomitant infection cases was 9574 per µl blood (median 5955; range 35 to 49,500). Presence of diurnal microfilaraemia needs further investigation.

Keywords: Concomitant infection, Filariasis, Malaria

INTRODUCTION

Naturally acquired concomitant infections of heterogenous pathogens in the same host are not uncommon. The association of human bancroftian filariasis and plasmodial species is one such example. In tropical and sub-

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tropical countries, where both these infections are widely distributed, interactions between these two parasites in the same host may influence clinical picture, pathogenicity and even epidemiology of the diseases they cause. In Orissa state of peninsular India, malaria[1,2] and filariasis[3] both are endemic, however, no information is documented on concomitant infections of causative agents in indigenous human population. During a longitudinal study on malaria from 1989 to 1991 this unique association of two heterogenous parasite species was encountered. Although preliminary observations made in this study were earlier documented in MRC annual report[4], the present paper describes in detail the results of the full study.

MATERIALS AND METHODS

The study population comprised 38,664 people living in 150 hamlets of Bisra PHC in Sundargarh district of Orissa state in eastern India. The demography and geographical information about the area have been described previously[1]. The study was conducted from 1989 to 1991. Thick and thin blood smears from febrile cases were taken during daytime using disposable lancets through weekly house-to-house visits by surveillance workers. Blood slides were stained with JSB-stain and first examined under 10x objective lens to find out microfilariae, followed by examination under oil immersion lens (100x) for detection of human plasmodia using Zeiss KF2 microscopes.

Cases of malaria and microfilaraemia were treated as per the recommended doses of the drugs prescribed by the national programme. All the patients showing concomitant infections were treated first for malaria followed by filariasis. Malaria parasite density (per microlitre blood) was estimated by counting parasites against 1000 leucocytes in thick smears based on the standard criterion of 8000 leucocytes per microlitre of blood. Microfilariae were counted per 10 µl blood.

RESULTS AND DISCUSSION

Of the total 51,448 blood smears taken in daytime during the three years study, 18,444 (35.84%) were positive for malaria parasites which comprised 3401 (18.44%) *Plasmodium vivax*, 14,524 (78.75%) *P. falciparum*, 156 (0.84%) *P. malariae* and 363 (1.97%) mixed plasmodial infections. Only 240 (0.46%) cases were positive for *W. bancrofti* microfilariae (mf). Of these 160 were frank mf cases, while 80 (33.3%) showed concomitant infections with malaria parasites viz. with *P. vivax* (14), *P. falciparum* (64), *P. malariae* (1) and *P. vivax* plus *P. falciparum* (1) (Table 1). Cases with concomitant infections were mostly prevalent during post-monsoon season apparently because of high malaria incidence during the period,

whereas frank mf cases were uniformly distributed.

Analysis of mf cases by age (Table 2) revealed that there was a gradual increase of prevalence in the age-group of 20-29. A decline in the age-group of 30-39 followed by a marginal increase in later age-groups was noticed. Malaria incidence in young children was very high (API 190.7) followed by a small drop in 5-19 age-group. The incidence were highest in 20-29 age-group but declined in the later age-groups. Concomitant infections were prevalent in a wide range of age-groups; the youngest case was a four year old female child and the oldest being 80 year old male. Sexwise distribution of concomitant infection indicated that males (61) were more affected than the females (19). The same trend was also observed in frank mf cases. In concomitant infection cases malaria parasitaemia was very divergent with a mean parasite density of 9574 per µl blood (median 5955; range 35 to 49,500), whereas mf density was low varying from 1 to 7 parasites per 10 µl blood. In most cases (90%) microfilariae were within the range of 1

Table 1. Incidence of malaria and filariasis in Bisra PHC (1989-91)

Total BSE	Malaria cases					mf cases only	Concomitant infection				
	Pv	*Pf*	*Pm*	Mix	Total		*Pf*+mf	*Pv*+mf	*Pm*+mf	*Pv*+*Pf*+mf	Total
51,448	3401	14,524	156	363	18,444	160	64	14	1	1	80

Pf — *P. falciparum*; *Pv* — *P. vivax*; *Pm* — *P. malariae*; mf — *W. bancrofti* microfilariae.

Table 2. Agewise distribution of filariasis and malaria in Bisra PHC

Age (yrs)	mf cases	mf cases/ 1000 pop./ year	Malaria cases	Malaria cases/1000 pop./year
≤ 4	2	0.2	2455	190.7
5-14	29	0.9	5391	171.2
15-19	22	1.8	2039	170.1
20-29	76	3.5	4377	203.2
30-39	42	2.6	2106	130.9
40-49	36	3.2	1190	105.9
≥ 50	33	3.1	886	82.2

to 4 per 10 µl blood. Similar observation was made in frank mf cases.

Reports on concomitant infection with bancroftian filariasis and plasmodial species are very few[5,6]. The area under study presents a meso- to hyper-endemic malaria situation where *P. falciparum* contributes nearly 80% of the total malaria cases. This is also true in concomitant infection in which *P. falciparum* contributed 81.25%. It was observed that concomitant infection cases were more prevalent in males than females. This may be due to the social and behavioural factors such as the females in tribal areas are more protected through proper clothing than males.

Most studies under laboratory conditions have indicated that interactions between malaria and a second parasite has either benign or a suppresive effect on malaria parasites[7,8]. Schimidt and Essinger[9] demonstrated that microfilaraemic infections in *Aotus trivergatus griseimembra* resulted in benign *P. falciparum* infection than in amicrofilaraemic monkeys. In the present study under natural conditions, this phenomenon holds good as highest malaria parasitaemia was within 50,000 per µl blood. Microfilaria parasite density was very low as in 72 (90%) cases parasite count varied from 1 to 4 per 10 µl blood. This may be due to the fact that slides were collected during daytime when low microfilariae density is expected. Although Indian *W. bancrofti* strains are widely known as nocturnal, presence of mf in peripheral blood during daytime is possibly due to diurnal overflow of microfilaraemia in cases having hyper-infection. There is therefore a need for further study on the periodicity of mf in this area.

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SHORT NOTES

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Studies on Clasper Movement of *Anopheles* Species

KANG WANMIN, CHEN HUAILU and XI YUNHUA

Keywords: *Anopheles*, Clasper movement, Induced mating

McDaniel and Horsfall[1] reported with photographic documentation, the first successful mating of *Aedes stimulans* and *Ae. vexans* in the laboratory. To anaesthetize mosquitoes CO_2 or chloroform was used, and then both males and females were affixed to the tops of fine needles. The male was placed on a white board with its ventral side up, while the female was made to touch the male end to end. A responsive male could inseminate two or more females. However, the rate of fertilization declined from 90, 85 and 50% in the first, second and third copulation respectively. According to McDaniel and Horsfall[1] there is a center for inhibition of copulatory activity located in the head of the male insect; probably in the suboesophageal ganglion. So males should be decapitated before induced copulation. An unsuccessful trial of induced copulation was made in *Culex pipiens* and *Anopheles quadrimaculatus*. Successful artificial copulation of *An. maculipennis* was carried out in the laboratory by Frizzi[2].

The technique of McDaniel and Horsfall[1] was slightly modified and a more

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practical method was established by Baker *et al.*[3] In this CO_2 or chloroform was used as an anaesthetic, a male mosquito was affixed with glue on to a microscope slide, and a female was held at an angle of 45^0 to the male. Copulation was observed under a dissecting microscope at 30x magnification. In this way, three geographical strains of *An. quadrimaculatus*, *An. freeborni*, *An. albimanus* and *An. earlei* were bred generation after generation in the laboratory. The method is widely adaptable to other *Anopheles* species. By the technique sterile eggs were obtained from crosses between (i) *An. freeborni* and *An. quadrimaculatus*; (ii) *An. maculatus* and *An. quadrimaculatus*; and (iii) *An. maculatus* and *An. freeborni*. In general, the technique is very useful for maintaining strains in the laboratory, making genetical crosses, and studying genetic isolating mechanisms, insecticide resistance, etc. Since then, Baker's method had been cited by many scientists while using induced copulation. Without a method to stimulate copulatory behaviour in mosquitoes, study of associated clasper movement would be difficult, if it is possible, to conduct. The paper reports results obtained on clasper movement in *Anopheles*.

Male clasper movement was first noted by Baker *et al.*[3] under a dissecting microscope while studying induced copulation. Later, Kanda and Oguma[4] observed differences in the number of clasper movement between species such as *An. sinensis*, *An. lesteri*, *An. engarensis*, *An. sineroides* and *An. koreicus*. These authors used the phrase "frequency of clasper movement" by which they meant the number of times the male clasper moved during one act of copulation.

Wanmin *et al.*[5] reported the clasper movements of 13 *Anopheles* species. The males remained quiescent for 2 to 15 s after the initiation of copulation, and then they began to move their claspers for 4.9 to 18.8 s at a time. There were obvious differences in the behaviour patterns of various species. The insemination rate of females hardly differed from that when the number of clasper movements were artificially limited to 0-13 times. According to the reports by Wanmin *et al.*[5], there was no significant difference in the number of clasper movements under various conditions including area of origin of the strain, temperature, age or recovery time after anaesthetization.

Kanda and Oguma[4] has reported that the number of clasper movements of the Engara strain of *An. sinensis* in Japan was 14.6 and this differed from *An. sinensis* in other districts which had an average of 8.0. Crosses confirmed that genetic separation existed between these strains. Therefore, the former strain was named *An. engarensis*. Wanmin *et al.*[6] noted, obvious differences between *An. anthropophagus* and *An. lesteri* (Japan) where

number of clasper movements were 7.0±0.2 and 8.2±0.3 respectively. However, *An. sinensis* from China and Japan were indistinguishable with scores of 8.5±0.2 and 8.2±0.3 respectively. No significant difference was observed between (i) *An. sinensis* and *An. changfus*[7]; (ii) *An anthropophagus*, *An. kiangsiensis* and *An. dazhaius*; (iii) *An. yatsushiroensis* and *An. xiaokuanus* (Table 1). Furthermore, Wanmin *et al.*[8] showed no significant difference between *An. kwnmingensis* and *An. liangshanensis*. Similar observations were made by Zhonghua *et al.*[9], that there was no overlap in the range of the number of clasper movements between *An. freyi* (China) with 58-89 and *An. koreicus* (Japan) with 40-50 times. All these studies agreed that the mosquitoes which were genetically separated were obviously different, in contrast to mosquitoes without genetic separation where differences are insignificant.

Wanmin *et al.*[6] has shown the possible differentiation of various sibling species. Yet, many doubts in mosquito classification are still to be clarified. Cross-mating and observations on sterility are the best methods for resolving these doubts, but obtaining live mosquitoes from different provinces or countries at the same time is not very easy. Therefore, the proposed technique of observing clasper movements is indeed a useful tool that can be applied as an auxiliary method in classification of sibling species for correcting misidentified species (Table 1) and confirming a species (Tables 2 and 3).

Table 1. Clasper movement of some of the mis-identified *Anopheles* species

Species	No. examined	Mean ± SE	Av. *p*-value
An. changfus	55	8.4±0.20	>0.05
An. sinensis	30	8.5±0.16	
An. dazhaius	32	6.9±0.19	
An. kiangsiensis	50	6.9±0.12	>0.05
An. anthropophagus	30	7.0±0.21	
An. xiaokuanus	30	8.4±0.36	>0.05
An. yatsushiroensis	30	8.4±0.28	
An. kwnmingensis	37	8.9±0.19	>0.05
An. liangshanensis	37	8.7±0.19	

Note: The identity of pairs was subsequently supported by fertility from crosses and homosequential chromosomes.

Table 2. Comparison of the number of clasper movement of *Anopheles* sibling species in China and Japan

Species	No. examined	Mean ± SE	Av. *p*-value
An. freyi (China)	32	69.4±1.48	
		58-89	<0.01
An. koreicus (Japan)		40-50	
An. anthropophagus (China)	32	7.0±0.21	<0.01
An. lesteri (Japan)	10	8.2±0.3	

Table 3. Variation in the number of clasper movement of new species identified by crosses

Species	No. examined	Mean±SE	Av. *p*-value
An. liangshanensis	37	8.7±0.19	<0.01
An. kweiyangensis	41	27.4±0.90	
An. engarensis	10	14.6±0.3	<0.01
An. sinensis	10	8.1±0.3	

According to Rattanarithikul and Green[10], Rattanarithikul and Harbach[11], *An. maculatus* was originally regarded as a single species, but now 12 species have been distinguished in Thailand, Philippines and India. Therefore, *An. maculatus* is now classified as a species group. According to Meng and Hanbin[12], there were 61 *Anopheles* species in 15 groups in China, some of these need to be clearly identified. If the number of clasper movements is added to the taxonomic methods for mosquito identification, it will be meaningful to make comparison in China and other parts of the world.

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Distribution of Indoor-Resting *Anopheles fluviatilis* in Human Dwellings and its Implication on Indoor Residual Spray

K. GUNASEKARAN, P. JAMBULINGAM and P.K. DAS

Keywords: *An. fluviatilis*, Malaria control, Residual spray, Resting behaviour

Apart from technical, administrative and social problems[1], bionomics and behaviour of the vector species are important obstacles to the interruption of malaria transmission by intradomicilary application of residual insecticides. In Koraput district of Orissa, indoor residual spray operations carried out by national programme have not been able to interrupt transmission and malaria continues to persist[2]. Systematic studies on the bionomics and behaviour of the major vector *Anopheles fluviatilis* were carried out to identify the causes for the persistence of malaria[3-7]. As a part of the study, the distribution pattern of this species in human dwellings was studied and the results are communicated in this paper.

The malaria problem in Koraput district and its physiographic divisions have been described elsewhere[2]. There are four distinct physiographic zones in the district. The seasonal pattern of malaria incidence, vector abundance and behaviour vary in Jeypore and Malkangiri zones. This study was carried out in a hill top village, Khandhaguda of Malkangiri zone, where *An. fluviatilis* has already been reported to be predominantly resting indoors, particularly in human dwell-

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ings[8]. The vector is however, exophilic in Jeypore zone[3]. Malkangiri zone is the southern part of the district (Koraput district) at an altitude of 150 m above the sea level. Resting collections were carried out in three human dwellings by hand catch method using aspirators in morning hours (0600 to 0900 hrs) and during early hours of night (1800 to 2100 hrs). The interior of the entire hut was searched including all hanging objects. In the night time, collections were made only from the veranda and exterior walls of the huts due to constraints of interfering with the privacy of the occupants. Marks were made on the walls according to the height from the floor and different sections/strata viz. below one foot, between one and two, two and three, three and four and above four feet. Collections were maintained separately according to different sites/wall heights in the hut. The data obtained were analysed using simple chi-square test. The study was carried out in the month of November (cold season), the period of peak prevalence of vector population and high malaria incidence[9].

In total, six day and night collections were made, 689 *An. fluviatilis* females were obtained during the morning hours. Comparison of the number of *An. fluviatilis* resting on different sites showed that walls were the most preferred resting site followed by roof. A considerable proportion of the collection was from hanging objects like umbrellas, ropes, bottles, clothes etc., as well as from objects kept on floor such as baskets, mudpots, gunny bags etc. (Table 1).

The number of *An. fluviatilis* obtained from different sections (height) of the walls was analysed. There was a significant positive correlation (r = 0.948; p = 0.014) between the number of *An. fluviatilis* collected and the height. Maximum number were collected from the height of four feet and above (Table 1) and it was significantly ($p<0.05$) higher than that obtained from the other sections of the wall. Similarly, the number collected between three and four feet

Table 1. Number and percentage of *An. fluviatilis* collected from human dwellings (morning hours)

Resting site	No. collected (n = 689)	%
Height from the floor (ft)		
< 1	5	2.30*
1-2	14	6.45*
2-3	25	11.52*
3-4	64	29.49*
> 4	109	50.23*
Total	217	39.31+
Roof	182	39.97+
Hanging objects	108	19.57+
Objects on floor	182	8.15+

*Percentage out of total collected on walls; +Percentage out of number collected.

was significantly higher than that obtained below this level. However, the number collected between two and three feet was not significantly (chi-square 2.817; p = 0.093) higher than that recorded between one and two feet and it was significant (chi-square 12.97; p<0.05) when compared with that of below one foot.

A total of 373 *An. fluviatilis* was collected during the early hours of the night. Out of this, 48.5% was captured on the walls, 32.5% from the roof of the veranda and entrance and 19.0% from the outside edges of the roof (Table 2) indicating a higher preference to walls for resting during night time also. The number of *An.*

Table 2. Number and percentage of *An. fluviatilis* collected from human dwellings (night hours)

Resting site	No. collected (n = 373)	%
Height from the floor (ft)		
< 1	5	2.76*
1-2	22	12.15*
2-3	26	14.36*
3-4	44	24.31*
> 4	84	46.41*
Total	181	48.53+
Roof (veranda)	121	32.44+
Roof (edges)	71	19.03+

*Percentage out of total collected on walls; +Percentage out of number collected.

fluviatilis resting on the walls showed a significant (r = 0.945; p = 0.015) positive correlation with the height following a similar pattern as observed during daytime resting.

The results indicate that *An. fluviatilis* preferred to rest on walls in human dwellings during both day and night time. However, a considerable proportion (27.72%) was found resting on unsprayable surfaces such as hanging objects and the objects kept on the floor. Resting was also noticed on exterior of the walls during night time. On the walls, more than 50% were collected at a height between 3 and 4 ft and above 4 ft. This observation was in contrary to the earlier report by Weeks[10], who concluded that in Rayagada zone of this district, 64% of the female were found resting on walls below 2 ft and only 3% preferred to rest on unsprayable surfaces. Weeks[10] pointed out that mud-plastering the floor and inaccessibility of the lower portion of the walls for spraying due to the household objects kept against it reduced the effect of residual insecticides. This may not be a problem in Malkangiri zone, as the present study indicates that only a small proportion of *An. fluviatilis* population rest on the lower portion of the walls. However, the proportion resting on unsprayable surfaces was much higher when compared to the earlier observation[10] and this population might escape from the effect of spray.

Moreover, the sprayed surfaces of the walls are mud-plastered after the spray operation[1] and this habit would make the spray ineffective on walls where maximum resting of *An. fluviatilis* was noticed. It was recently reported that in tribal villages where mud-plastering was deferred for sometime after insecticide spray, the reduction of malaria prevalence was 72% and where there was mud-plastering the reduction was only 49%. Though, it is clear that mud-plastering reduces the efficiency of the spray, it can not be prevented as it is customary for the tribal people[11]. In addition, the outer surfaces of the walls are not sprayed as insecticide will be degraded and washed off by sunlight and rains. As a result, mosquitoes resting on exterior surface of the walls would be free from insecticide contact. All these factors led to conclude that though *An. fluviatilis* is predominantly endophilic in the study zone, indoor residual spraying have limited impact due to socio-cultural practice of the tribals and distribution pattern of the indoor resting mosquitoes. In this situation, alternate strategies, such as personal protection measures using impregnated bednets could be encouraged.

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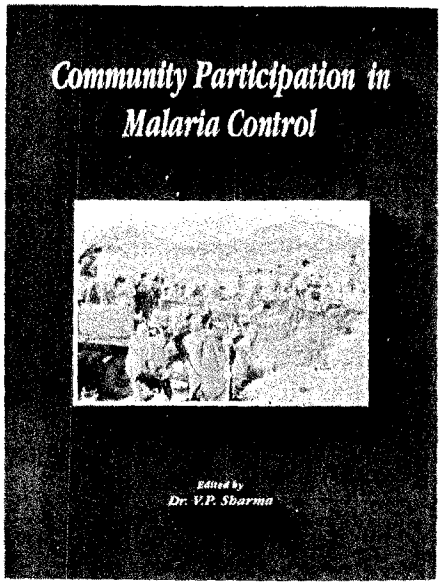

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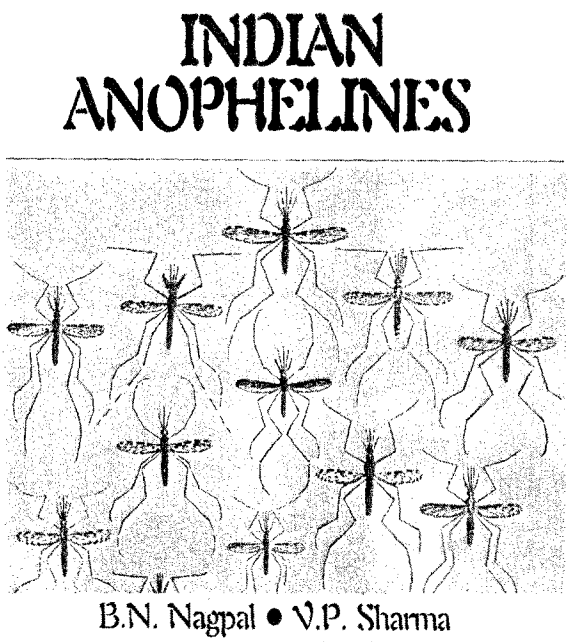

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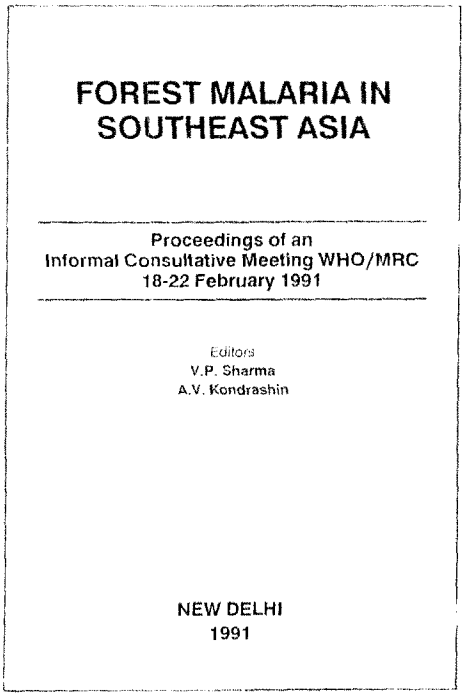

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