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Letter to the Editor

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Comparative Toxicity of Certain Mosquitocidal Compounds to Larvivorous Fish, *Poecilia reticulata*

P.K. MITTAL, T. ADAK and V.P. SHARMA

Toxicity of certain mosquitocidal compounds (both larvicides and adulticides) to the larvivorous fish *Poecilia reticulata* was determined in the laboratory. Among the various chemical insecticides tested, the synthetic pyrethroid deltamethrin was most toxic to fish (LC50 = 0.016 ppm), while the organophosphorus insecticide abate was least toxic (LC50 = 34 ppm). The bioinsecticides Spherix (*Bacillus sphaericus*) and Bactoculicide (*Bacillus thuringiensis* H-14) showed highest safety for the fish (LC50 > 1000 mg/litre). Integrated use of larvivorous fish and bioinsecticide in vector control has been suggested.

Keywords: Insecticides, Mosquitocidals, Mosquito larvae, *Poecilia reticulata*, Toxicity

INTRODUCTION

The larvivorous fish *Poecilia reticulata* (Peters), commonly known as guppy, is a widely distributed fish in India[1]. This fish can tolerate a certain degree of organic pollution and has been reported to be well established in sullage water[2] and other habitats[3]. The fish has been extensively used in Kheda District, Gujarat, under the bioenvironmental control of malaria vectors[4]. The efficacy of guppy fish in the control of *Culex* in polluted waters in rural areas of Delhi has also been reported[5]. The fish alone, however, has certain limitations and

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therefore integrated control of mosquitoes using larvivorous fish and conventional mosquitocidal compounds (both larvicides and adulticides) is often recommended[6,7]. This however requires compatibility between these agents. In the past few years, several new mosquitocidal compounds have been evaluated at the Malaria Research Centre. It is therefore important to know their toxicity against larvivorous fish before they can be used in the integrated vector control programme. This paper reports the comparative toxicity of some of these new compounds and also the existing ones against *Poecilia reticulata*. For comparison of the safety margin of larvicides, the susceptibility of *Anopheles stephensi* and *Culex quinquefasciatus* mosquito larvae was also determined to the same larvicides which were tested against the larvivorous fish.

MATERIALS AND METHODS

The larvivorous fish used for this study were collected from drains of Modi Nagar steel factory in Ghaziabad District, near

Table 1. Acute toxicity of certain mosquitocidal compounds to the larvivorous fish, *Poecilia reticulata*

Compound	Class	Lethal concentrations (ppm, ai)		χ^2 (df)	Regression equation
		LC50	LC90		
p,p' - DDT	O-Cl	0.052 (0.042-0.064)	0.14	6.5(3)	$Y = 3.02+2.76x$
Gamma-HCH	O-Cl	0.18 (0.151-0.214)	0.446	2.27(2)	$Y = 0.96+3.22x$
Cypermethrin	S-P	0.092 (0.0739-0.114)	0.27	5.39(3)	$Y = 2.31+2.79x$
Deltamethrin	S-P	0.016 (0.012-0.021)	0.052	2.04(3)	$Y = 1.96+2.52x$
K-othrine (Deltamethrin 2.5% Flow)	S-P	18.0 (14.3-22.6)	46.0	1.17(3)	$Y = 1.15+3.07x$
Temephos	O-P	34 (27.64-41.8)	100.0	2.75(2)	$Y = 0.76+2.77x$
Fenthion	O-P	6.6 (2.29-2.94)	4.5	5.1(2)	$Y = 2.59+5.82x$
Spherix (*B. sphaericus*-101)	Bioinsecticide >1000 mg/l (No mortality was observed up to 48 h)				
Bactoculicide (*B. thuringiensis* H-14)	Bioinsecticide >1000 mg/l (No mortality was observed up to 48 h)				

O-Cl = Organochlorine; O-P = Organophosphorus; S-P = Synthetic pyrethroids.

Delhi, and were kept in plastic tubs containing stored tap water. The fish until use were fed on fish food and mosquito larvae. Larvae of *Anopheles stephensi* and *Culex quinquefasciatus* (wild strain from Delhi) were reared in the mosquito colony being maintained at Malaria Research Centre. The insecticides, *p,p'*-DDT and γ-HCH were of analytical grade. Cypermethrin (25% EC), deltamethrin, temephos, Fenthion (Technical) and K-othrine (Deltamethrin 2.5% Flow) were obtained directly from the manufacturers. The bioinsecticide preparations Spherix *(B. sphaericus)* and Bactoculicide *(B. thuringiensis* H-14) were produced in Russia and supplied by M/s. Chemicals International Ltd., Delhi.

Methods used for determining the susceptibility of mosquito larvae were the same as described by WHO[8]. The toxicity of insecticides against the fish was determined by the procedure already described[9]. In short, ten guppies (2.5-3.0 cm in length) were added to 2 litres of water taken in 5-litre beakers. The test concentrations were prepared by dissolving 1 ml of serially diluted stock solution. The stock solutions with technical grade insecticides were prepared in ethanol, while the emulsifiable and liquid formulation and wettable powders were dissolved in water. There were four replicates for each concentration and the control. Observations were recorded after 24-h exposure and the corrected per cent mortalities were determined by using Abbott's formula[10]. Lethal concentration (LC50 and LC90) values of different insecticides were determined from the log-Probit regression lines and confidence limits for LC50 were determined by the method of Litchfield and Wilcoxon[11].

RESULTS AND DISCUSSION

Table 1 shows the acute toxicity of different insecticides to the larvivorous fish *Poecilia reticulata*. Results revealed that among the different chemical insecticides, the synthetic pyrethroid deltamethrin was most toxic to the fish while the organophosphorus mosquito larvicide, temephos, was least toxic. Among the two pyrethroids, deltamethrin was more toxic (LC50 = 0.016 ppm) than cypermethrin (LC50 = 0.092 ppm). However, the water-based flowable formulation of deltamethrin (K-othrine 2.5% Flow) was much less toxic (LC50 = 18 ppm) probably due to adsorption of active ingredients on the formulation particles and thus low solubility of active ingredients in water. A similar observation was also made with the mosquito fish *Gambusia affinis*[9]. Of the two organochlorine insecticides, DDT was more toxic (LC50 = 0.052 ppm) than γ-HCH (LC50 = 0.18 ppm) and of the two organophosphorus insecticides, fenthion was more toxic (LC50 = 2.6 ppm) than temephos (LC50 = 34 ppm). The bioinsecticides Spherix *(Bacillus sphaericus)* and Bactoculicide *(Bacillus thuringiensis* H-14) were found safe to the fish even at a very high concentration (Table 1).

Table 2 shows the LC50 values of four larvicides against mosquito larvae of *An. stephensi* and *Culex quinquefasciatus* and also the fish safety factor (FSF). The

Table 2. LC50 values and fish safety factor of four larvicides

Insecticide	LC50 ppm, active ingredients (95% confidence limits)			
	An. stephensi	*Cx. quinquefasciatus*	FSF* over *An. stephensi*	FSF over *Culex*
Temephos (Tech.)	0.016 (0.014-0.017)	0.0022 (0.019-0.0024)	237	1551
Fenthion (Tech.)	0.018 (0.015-0.012)	0.009 (0.00075-0.011)	22	32.4
Spherix *(B. sphaericus)*	0.19 (0.135-0.239)	0.056 (0.046-0.067)	>1219†	>4545†
Bactoculicide *(B. thuringiensis* H-14)	0.16 (0.123-0.207)	0.062 (0.049-0.078)	>1087†	>3704†

*FSF (Fish safety factor) = LC05 fish/LC95 larvae; †Since there was no mortality of the fish at the concentration up to 1000 mg/l, the fish safety factor of the bioinsecticides was roughly estimated with this concentration.

FSF is defined as the ratio of the values LC05 fish/LC95 larvae[12]. Of the four mosquito larvicides, two bioinsecticides, viz. Bactoculicide *(B. thuringiensis* H-14) and Spherix *(B. sphaericus)*, had the highest safety margin for the fish (Table 2). Temephos had a lower margin of safety for fish (FSF over *An. stephensi* and *Cx. quinquefasciatus* being 237 and 1551 respectively) even though it was most toxic against mosquito larvae (LC50 values against *An. stephensi* = 0.016 ppm and *Culex quinquefasciatus* = 0.0022 ppm). But fenthion had the lowest margin of safety for fish (FSF over *Culex quinquefasciatus* and *An. stephensi* being 22 and 32 respectively). The bioinsecticides *B. thuringiensis* H-14 and *B. sphaericus* have been reported to be safe to non-target organisms including larvivorous fish[13-15].

Keeping in view the fish safety factor of the four larvicides tested in our study, the use of the bioinsecticides Bactoculicide *(B. thuringiensis* H-14) and Spherix *(B. sphaericus)* can be recommended in conjunction with larvivorous fish *P. reticulata* against filaria vector and also against malaria vector *(An. stephensi)* and mosquito nuisance in urban areas.

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Correlation of Malaria Endemicity with *An. culicifacies* Sibling Species Composition and Malaria Antibody Profile in District Allahabad (U.P.)

S.N. TIWARI, ANIL PRAKASH, S.K. SUBBARAO[a], ARTI ROY[a], HEMA JOSHI[a] and V.P. SHARMA[a]

Entomological, parasitological and serological surveys were conducted between October 1989 and November 1990 in 27 villages (population 33,250) belonging to three topographically different areas of district Allahabad, viz. Gangapar, Doaba and Yamunapar. A good correlation existed in all the three areas between malaria incidence vis-a-vis *An. culicifacies* sibling species composition and malaria antibodies titre in the populations. In Gangapar and Doaba villages, *An. culicifacies* densities were low and the proportions of vector species A and C were much less than that of species B, the non-vector species. Low endemicity of malaria was supported by low antibody titres observed in the population. In contrast, in Yamunapar villages *An. culicifacies* densities were high, species A and C together were almost equal to species B, and malaria incidence as well as antibody titre were high. Based on these observations, district Allahabad, Uttar Pradesh, could be divided into two zones, low malaria transmission zone — Gangapar and Doaba areas and high malaria transmission zone — Yamunapar area.

Keywords: *An. culicifacies*, Malaria antibody, Sibling species, Stratification

INTRODUCTION

Anopheles culicifacies is the major vector of malaria in the Indian subcontinent. This species is a complex of 4 sibling species[1]. Extensive surveys carried out in different parts of the country to study the distribution and composi-

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tion of sibling species have shown the prevalence of species B almost throughout the country wherever *An. culicifacies* is encountered[2]. Species A is found from north to south with high proportions in north, which gradually decline towards south. Sibling species C is found from west to east and species D is found in northwest and central India. In central India and also in Sathanur dam area in south India, all four sibling species are sympatric. In northern India, it has been shown that there is a relationship of malaria incidence to sibling species prevalence by identifying the predominance of species A in high malaria incidence areas and that of species B in low incidence areas[3]. Vector incrimination studies using immunoradiometric assay have shown that species A, C and D harbour *Plasmodium vivax* and *P. falciparum* sporozoites[4,5]. That only one specimen of species B was found with low numbers of *P. vivax* sporozoites shows that species B has a minor role, if at all, in the transmission of malaria in northern India.

Differences in the vectorial potential among sibling species and their distribution pattern in the country have explained the epidemiological differences observed in the area of *An. culicifacies* distribution. This suggested that areas could be stratified on the basis of the distribution of sibling species. With this objective a study was initiated in rural areas of district Allahabad, U.P.

In this district, *An. culicifacies* is the principal malaria vector. For this study, *An. culicifacies sensu lato* densities, sibling species composition and antibody titre against malaria parasite antigens and malaria incidence in the population were monitored and the results are reported in this paper.

MATERIALS AND METHODS

District Allahabad located in southeastern part in Uttar Pradesh lies between the latitudes 24° 47' and 25° 47' N and longitudes 81° 19' and 82° 29' E. Two rivers, the Ganga and the Yamuna, traverse across the district and meet in Allahabad city, dividing the entire district into three parts, viz. Gangapur, Doaba and Yamunapar (Fig. 1). Tons and Belan are the two other important rivers of this district. There are a number of canals, ponds, streams, stone quarries, tubewells and wells in the district. The climate of the district is characterised by long hot summer, May and June being the hottest months. The southwest monsoon breaks towards the end of June and lasts till September. Winter season extends from November to February. The population of the district is 49,09,919 (1991 census) distributed over 9 tehsils and 28 primary health centres (PHCs). There are 3 tehsils and 11 PHCs in Gangapar area, 3 tehsils and 8 PHCs in Doaba area and the remaining 3 tehsils and 9 PHCs in Yamunapar area.

A total of 27 villages (Fig. 1), 5 belonging to Gangapar, 9 to Doaba and 13 to Yamunapar tracts representing different epidemiological and ecological profiles, were surveyed for incidence of malaria, and *An. culicifacies* sibling spe-

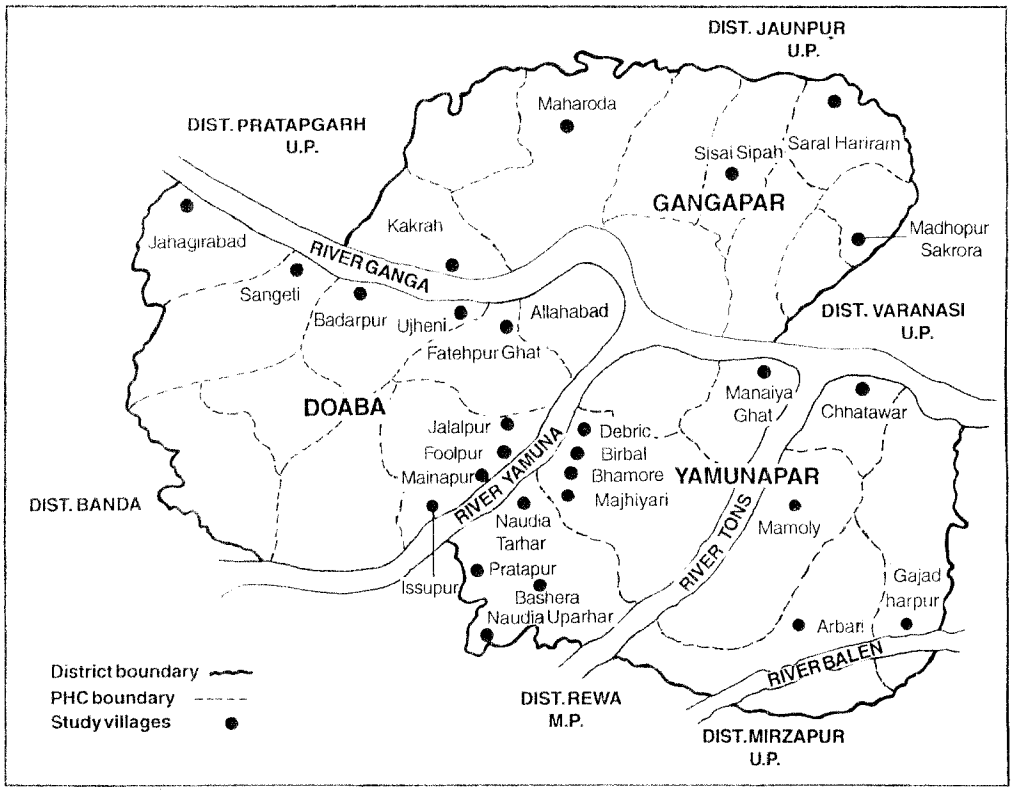

Fig. 1 : Map of District Allahabad showing study villages

cies composition between October 1989 and November 1990. Each village was surveyed twice during the study period. Seroepidemiological surveys were, however, done only once in all the study villages between February and August 1990.

Entomological investigations

Density determination: Indoor resting populations of *An. culicifacies* were collected from all study villages between 0600 and 0800 hrs both from human dwellings (HD) and cattlesheds (CS) by suction tube method and per man hour density (PMHD) was determined. Total mosquitoes were also collected by pyrethrum space spray method from HD in all villages (4 HD in each village) to calculate the density per structure. All semigravid female *An. culicifacies* specimens were separated and processed for identification of sibling species.

Sibling species identification: Ovaries from all semigravid females of *An. culicifacies* were removed and fixed im-

mediately in modified Carnoy's fixative (glacial acetic acid : methanol :: 1 : 3) and stored in a refrigerator till further processing. Polytene chromosome preparations from fixed ovaries were made according to Green and Hunt (1980)[6] and sibling species were identified with the help of diagnostic inversion karyotypes[7,8].

Species A — X+a+b $2+g^1+h^1$, $2+i^1/i^1$

Species B — Xab $2g^1+h^1$

Species C — Xab $2+g^1h^1$

Host feeding preference of sibling species: Blood from abdomens of semigravid females was smeared on filter paper (no. 1) for detecting the host source. Each blood smear was assayed against bovine and human antisera by countercurrent immunoelectrophoresis[9]. Ovary and blood smear from each mosquito were correspondingly numbered in order to know the host feeding preference of sibling species.

Parasitological investigations

Mass blood surveys were carried out in all selected villages. Blood smears after staining with JSB stain were examined under the microscope for the presence of malaria parasite.

Serological investigations

Mass blood surveys were conducted between February and August 1990. A drop of blood from each subject was collected on Whatman no. 3 filter paper strip in all study villages for estimating antibodies against malaria parasite antigens. Filter paper strips were stored in a refrigerator till further processing. The antimalarial antibody titre was estimated against a nanomer peptide derived from ring-infected erythrocyte surface antigen by enzyme-linked immunosorbent assay (ELISA) method[10].

RESULTS AND DISCUSSION

A total of ten anopheline species were collected from the study villages during our surveys (Table 1). *An. subpictus* was the predominant species followed by *An. culicifacies* and *An. annularis* in all the three areas. These three species together were accountable for more than 96% of total anophelines collected from study areas. Among the three areas, the highest number of *An. culicifacies* was collected from Yamunapar area (136/village), followed by Doaba (89.4/village) and Gangapar area (20/village). *An. culicifacies* per man hour densities, average number of mosquitoes collected per structure (from pyrethrum space spray collections) and sibling species composition are given in Table 2. As the pattern remained the same in the two collections, i.e. one in transmission and the other in non-transmission period, data were pooled (Table 2). Per man hour densities (PMHD) of *An. culicifacies* were lowest in Gangapar villages (0-8.5), highest in Yamunapar villages (35-59), whereas in villages of Doaba region the densities ranged from 2.3 to 24.5. More numbers were collected from cattlesheds than from human dwellings. The pattern of *An. culicifacies* density per structure was similar to that of PMHD ob-

Table 1. Prevalence of different anophelines in District Allahabad (U.P.)*

Area (No. of villages)	No. anophelines collected from human dwellings with pyrethrum space spray										Total
	An. cu	*An. st*	*An. su*	*An. pa*	*An. an*	*An. ac*	*An. ni*	*An. va*	*An. te*	*An. ba*	
Gangapar (5)	100 (2.1)	0 (0)	4541 (96.2)	19 (0.4)	39 (0.8)	1 (0.02)	0 (0)	61 (1.3)	0 (0)	0 (0)	4761 (100)
Doaba (9)	805 (34.3)	2 (0.08)	1369 (58.3)	43 (1.8)	90 (3.8)	2 (0.08)	4 (0.2)	30 (1.3)	1 (0.04)	1 (0.04)	2347 (100)
Yamunapar (13)	1772 (14.0)	19 (0.2)	10194 (80.6)	47 (0.4)	484 (3.8)	34 (0.3)	1 (0.008)	96 (0.8)	0 (0)	4 (0.03)	12651 (100)

* Data from two surveys carried out between Oct 1989 and Nov 1990; *An. cu—An. culicifacies; An. st—An. stephensi; An. su—An. subpictus; An. pa—An. pallidus; An. an—An. annularis; An. ac—An. aconitus; An. ni—An. nigerrimus; An. va— An. vagus; An. te— An. tessellatus; An. ba—An. barbirostris.* Figures in parentheses show per cent composition of a species out of the total number of anophelines collected from that area.

Table 2. *An. culicifacies* densities and sibling species composition in District Allahabad (U.P.)

Area	Per Man Hour Density*		Density* per structure (HD only)	No. examined for sibling species	Sibling species composition		
	HD	CS			A (%)	B (%)	C (%)
Gangapar	2.2	4.6	2.3	32	4 (12.5)	21 (65.6)	7 (21.9)
Doaba	10.3	22.1	11.2	267	26 (9.7)	236 (88.4)	5 (1.9)
Yamunapar	16.3	39.8	17.1	520	192 (36.9)	274 (52.7)	54 (10.4)

*From the two surveys done between Oct 1989 and Nov 1990; *Pyrethrum space spray collection; HD — Human dwellings; CS — Cattlesheds.

served in the three areas, i.e. lowest in Gangapar (2.3), highest in Yamunapar (17.1) and intermediate in Doaba (11.2). The higher vector densities in Yamunapar villages were probably due to the availability of innumerable additional breeding grounds in the stone quarries in these villages.

In all the three areas, species A, B and C were sympatric. As the densities were very low in Gangapar villages, only 32 *An. culicifacies* were identified for sibling species. Species B was the predominant species in all the areas — 88% in Doaba area, 65.6% in Gangapar and 52.7% in Yamunapar. The highest proportion of species A (37%) was found in Yamunapar villages. Only a few specimens of species C were identified in Gangapar and Doaba areas while this species constituted 10% in Yamunapar villages.

Only in Yamunapar area, 6 specimens of species A and one of species C were found with human blood. All other specimens had bovine blood (Table 3).

The slide positivity rate varied: 1.8 in Gangapar, 2.8 in Doaba and 8.8 in Yamunapar. A similar trend was observed in the case of slide falciparum rate (Table 4). When parasitological data in respect of children between 1 and 10

Table 3. Bloodmeal analysis of *An. culicifacies* sibling species

Area	Species A		Species B		Species C	
	No. tested	HBI	No. tested	HBI	No. tested	HBI
Gangapar	4	0.0	21	0.0	7	0.0
Doaba	26	0.0	236	0.0	5	0.0
Yamunapar	172	0.035	220	0.0	35	0.03

HBI — Human blood index.

Table 4. Malaria incidence in district Allahabad (U.P.)*

Area	Population	BSE	Total positive	No. positive for			SPR	SfR	PI
				Pv	*Pf*	Mix			
Gangapar	9000	930	17	7	10	0	1.8	1.1	1.9
Doaba	11750	1309	36	4	32	0	2.8	2.4	3.1
Yamunapar	12500	2048	180	38	142	0	8.8	6.9	14.4

*Cumulative figures of two surveys done between Oct 1989 and Nov 1990; BSE — Blood smears examined; *Pv* — *P. vivax*; *Pf* — *P. falciparum*; Mix — *P. vivax* + *P. falciparum*; SPR — Slide positivity rate; SfR — Slide falciparum rate; PI — Parasite incidence.

years were analyzed, child parasite rates (CPR) of 1.5, 1.7 and 5.0 were recorded in Gangapar, Doaba and Yamunapar villages respectively. In the case of infants below 1 year, no malaria-positive case was recorded from Gangapar and Doaba villages, whereas in Yamunapar, 3 infants were found positive out of 39 examined (IPR 7.7). Two of them were positive for falciparum malaria and one was positive for *P. vivax* (Table 5).

Antimalarial antibody titres expressed in terms of ELISA OD values obtained for the populations belonging to the three zones of the district are given in Table 6.

Table 5. Malaria incidence in infants (below 1 yr) and children (1-10 yrs) of District Allahabad*

Area	Infants (below 1 yr)				Children (1-10 yrs)			
	No. examined	No. (+)ve	Parasite rate (IPR)	Falciparum rate (IfR)	No. examined	No. (+)ve	Parasite rate (CPR)	Falciparum rate (CfR)
Gangapar	25	0	0.0	0.0	406	6	1.5	1.0
Doaba	20	0	0.0	0.0	630	11	1.7	1.4
Yamunapar	39	3	7.7	5.1	862	43	5.0	2.9

*Cumulative figures of two surveys done between Oct 1989 and Nov 1990.

Table 6. Malaria antibodies titres against a synthetic peptide in population of Allahabad district (U.P.)*

Area	ELISA O.D. values†					All age groups
	0 - 5 yrs	6 - 10 yrs	11 - 20 yrs	21 - 40 yrs	>40 yrs	
Gangapar	0.161 ± 0.099 (118)	0.198 ± 0.118 (138)	0.184 ± 0.103 (128)	0.217 ± 0.123 (87)	0.210 ± 0.107 (63)	0.194 ± 0.11 (534)
Doaba	0.169 ± 0.121 (199)	0.184 ± 0.127 (233)	0.210 ± 0.135 (165)	0.182 ± 0.144 (147)	0.250 ± 0.112 (73)	0.119 ± 0.12 (817)
Yamunapar	0.725 ± 0.193 (233)	0.721 ± 0.204 (280)	0.694 ± 0.182 (244)	0.732 ± 0.177 (235)	0.719 ± 0.186 (113)	0.718 ± 0.18 (1105)

*Only one survey between Feb and Aug 1990; †Mean ± SD value. Figures in parentheses are numbers tested.

Mean OD values for the three zones were as follows: Gangapar, 0.19 ± 0.11; Doaba, 0.119 ± 0.12; and Yamunapar, 0.718 ± 0.18. Since the areas with ELISA OD value of less than 0.3 were considered as low endemic[10], Yamunapar with 0.718 OD can be categorized as highly endemic. When antibody titres for different age groups were analyzed, no age-related increase in antibody titre was found. Ramesh Kumar *et al.*[11] also did not observe any age-related increase in antibody titre in Ghaziabad (U.P.). However, in Hant-Ogone, which is highly endemic for malaria, the cumulative effect on antibody titre due to repeated exposures to malaria infection had been reported[12].

Malaria incidence was low in Doaba and Gangapar villages and was relatively high in Yamunapar. Seroepidemiological surveys also indicated that there was a low level of transmission in the two areas as the antibody titres were well below 0.3 in Gangapar and Doaba villages. In all the areas *An. culicifacies*, an established vector, was present. The sibling species composition, however, varied in the three areas. Among the 3 species present in this area, only two species A and C are considered as vectors[4,5].

In Yamunapar area, *An. culicifacies* densities were relatively higher. *An. culicifacies*, which is predominantly a zoophagic species, attains its importance as a vector in areas where high densities are observed. In Yamunapar, incidence of malaria was high, the antibody titre was high and 47% of *An. culicifacies* population comprised species A and C. And it is in this area that 3.5% of species A and 3.0% of species C were found fed on human blood. These findings suggest that species A and C were responsible for the active malaria transmission observed in Yamunapar villages.

This study by establishing a relationship between sibling species prevalence and malaria incidence confirmed earlier findings that in western U.P. where species A and B are sympatric with the predominance of species A, malaria incidence is high and in eastern U.P. where almost totally species B is prevalent, malaria incidence is either absent or very low[3].

Based on our study, district Allahabad could be divided into two distinct zones: (1) Gangapar and Doaba areas—a zone with low malaria transmission; and (2) Yamunapar—a zone having high malaria transmission. This classification may help mobilize limited resources to control malaria in an area which has a serious malaria problem. Based on this stratification, chemotherapy alone is recommended in Gangapar and Doaba area to treat the few cases; insecticidal spray is not necessary. In Doaba areas, where densities are relatively higher than in Gangapar villages, suitable environmental methods to control vector populations could be introduced. In Yamunapar villages, where incidence is high in addition to environmental measures, a judicious use of effective insecticides is recommended to prevent epidemics.

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Field Trial of Esbiothrin-Impregnated Rope in Ramgarh Village, Dadri PHC, District Ghaziabad (U.P.)

M.A. ANSARI and R.K. RAZDAN

A village-scale field trial was carried out to assess the operational feasibility and efficacy of smouldering 250 ppm esbiothrin-impregnated rope in repelling mosquitoes and its impact on transmission of malaria. Entomological monitoring revealed that smoke from smouldering rope resulted in 67.2-97.9% reduction of *An. culicifacies* densities in human and mixed dwellings. The reduction was obviously due to diversion of *An. culicifacies* population to cattlesheds where ropes were not burnt. The impact was more pronounced when indoor mosquito landing rate on human bait was compared with that of experimental and control areas. This was also reflected in reduced anthropophilic index and curtailment of malaria transmission in experimental area.

Keywords: *An. culicifacies*, Esbiothrin rope, Malaria control, Mosquito repellent

INTRODUCTION

The use of repellent oil, cream, insecticide-impregnated coil or mats is quite common in urban areas for prevention of mosquito bites. The rural populations belonging to low socio-economic group and living in unaccessible area, particularly tribals, continue to suffer from malaria because of nonavailability of simple and cheap methods of personal protection. Hence, an innovative indigenous method of personal protection was developed to repel mosquitoes. Jute rope was impregnated with 80 ppm deltamethrin and allowed to smoulder

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in open living rooms from dusk to dawn. The smoke from smouldering rope provided more than 90% protection from *An. culicifacies* bites but did not provide comparable protection from other mosquito species particularly against *Culex* spp[1]. Subsequently efforts were made to improve the efficacy of impregnated rope by replacing deltamethrin with esbiothrin. The replacement enhanced protection both against *An. culicifacies* and *Culex quinquefasciatus*[2]. In view of the promising results a small-scale field trial was carried out in Ramgarh village to evaluate the operational feasibility and efficacy of smouldering esbiothrin-impregnated rope in repelling mosquitoes and reducing the transmission of malaria. The results of this study are reported in this paper.

MATERIALS AND METHODS

Esbiothrin[@] is an improved isomeric composition of allethrin series and essentially consists of esters of chrysanthemic acid and allethrolone alcohol. The compound is commonly used in coils and mats marketed in India. Jute rope (0.009 m diam.) was selected because of its uninterrupted burning quality and easy availability in rural areas. Ramgarh village was divided into two sections. In one section (RC), plain rope was burnt and in the other section (RE), esbiothrin-impregnated rope was burnt only in human and mixed dwellings. Populations of experimental and control sections of Ramgarh village were 514 and 623 respectively. Jadaonpur village (JC) was taken as another control where no rope was burnt. The distance between Ramgarh and Jadaonpur villages is about 20 km. The rope was impregnated with 250 ppm esbiothrin as this dose was found effective in repelling *An. culicifacies* and also because of limited supply of esbiothrin. An iron mesh cylinder was used to cover the rope while burning as a preventive measure against fire. Ropes were distributed along with iron mesh cylinders to the head of family to use it in every room of human and mixed dwelling from dusk to dawn. Good supervision was maintained to ensure burning of rope in each unit from 1800 to 0600 hrs. Weekly monitoring of *An. culicifacies* day-time resting density in human and mixed dwellings and cattlesheds was carried out in two sections of Ramgarh and Jadaonpur villages. Indoor landing rate of mosquitoes on human bait was observed on fortnightly basis as described by Ansari *et al*[2]. Blood smears from freshly engorged *An. culicifacies* females were prepared on filter papers to determine the anthropophilic index by microdot ELISA technique during the transmission period[3].

Study sites

Ramgarh village of Dadri PHC, Distt. Ghaziabad (U.P.), was selected because

[@]The chemical name of esbiothrin is (±)-3-allyl-2-methyl-4-oxocylopent-2-enyl(+)-*trans*-chrysanthemate. The technical sample of esbiothrin was obtained through the courtesy of M/s. Roussels Pharmaceuticals India Ltd.

of the high density of *An. culicifacies* and persistent malaria transmission. It is located on Dadri and Bulandshahar highway southeast of Delhi at a distance of about 40 km from Delhi. The village has a total population of 1137, living in 76 houses and 79 mixed dwellings. There are about 80 cattlesheds made of mud walls and thatched roofs. Permanent mosquito breeding places are drains, stagnant irrigation channels, ponds, wells, pools and pits and minor drains inside the village.

Jadaonpur village in Dhaulana PHC, Distt. Ghaziabad (U.P.), was selected as a control village. The village is located on Massuri and Gulawthi road southeast of Delhi at a distance of about 35 km from Delhi. The village has a population of 693 living in 93 human and 56 mixed dwellings. There are 84 cattlesheds.

RESULTS AND DISCUSSION

The density of *An. culicifacies* in human and mixed dwellings was pooled together. The monthwise average density of cattlesheds and human and mixed dwellings in experimental (RE) and control section of Ramgarh (RC) and Jadaonpur (JC), along with indoor average mosquito landing rate on human baits, is given in Table 1. The table shows that the pre-experiment density was more or less the same in both RE and RC areas. Daily smouldering of esbiothrin rope in living rooms reduced the daytime resting densities of *An. culicifacies* in RE area in successive months. The reduction (%) in the RE and (JC) areas, monthwise, were as: August – 67.2 (1.5); September – 81.1 (26.0); October – 77.18 (39.3); November – 77.5 (22.6); December – 86.6 (0.0); January – 97.9 (56.5); February – 94.7 (13.3); and March 92.12 (0.0). In fact, there were 31.0 and 22.5% increase in the density of *An. culicifacies* in the months of October and March respectively. A marginal reduction of *An. culicifacies* density was also observed in RC area where untreated rope was burnt in human and mixed dwellings. This was quite expected as smoke alone from untreated rope provided 30-40% protection[2]. The reduction in day-time resting densities of *An. culicifacies* in human and mixed dwellings in RE and RC areas particularly after smouldering the rope may be attributed to the fact that this species is basically zoophilic and the smoke from smouldering rope in human and mixed dwellings would have repelled the species to take shelter in cattlesheds where no rope was burnt. There was an increase of 2.27, 2.17, 2.3 and 1.8 times in the densities of *An. culicifacies* in RE area as against 1.9, 2.0, 2.4 and 1.5 in RC area during August, September, October and November respectively in comparison to pre-experiment density of this species. No such increase in resting population of this species was observed in cattlesheds of JC area where no rope was burnt (Table 1). P values were greater than 0.05 when the MHDs of human and mixed dwellings were compared with corresponding MHDs of RC and JC areas. Similarly P values were less than 0.05 when the MHD of cattlesheds was compared with RC and JC areas during the post-operative period, suggesting thereby that there was a significant shifting of

Table 1. Entomological indices of Ramgarh and

Year/ Month	MHD of *An. culicifacies*						Indoor no. of mosquitoes landed/ bait/night					
	Human and mixed dwelling			Cattlesheds			*An. culicifacies*			*An. annularis*		
	RE	RC	JC	RE	RC	JC	RE	RC	JC	RE	RC	JC
Pre-experimental months												
May 90	192.6	–	75.85	90.0	–	93.7	–	–	–	–	–	–
Jun	75.5	–	70.12	112.0	–	166.7	–	–	–	–	–	–
Jul	104.75	150	46.0	129.0	146.0	86.5	15	15.5	20.0	0.5	0	0.5
Post-experimental months												
Aug	34.3	98.5	45.3	294.0	280.0	63.2	0	13.7	15.2	0	1.7	2.2
Sep	19.7	52.2	34.0	280.0	299.3	53.0	0	5.2	7.2	0	1.7	2.5
Oct	23.9	80.2	28.0	301.0	362.0	56.5	0	8.5	9.5	0	0.7	1.2
Nov	23.5	65.6	35.6	236.0	225.0	66.7	0.7	6.2	7.5	0	8.2	9.2
Dec	14.0	67.2	59.6	135.0	124.2	105.5	0	18.7	19.5	0	8.2	8.7
Jan 91	2.13	24.1	20.2	78.0	76.4	39.66	0	7.0	8.5	0	0.2	0.7
Feb	5.5	57.1	39.0	69.7	59.0	87.25	0	4.2	4.7	0	0.2	0.7
Mar	8.25	46.6	59.4	65.0	51.0	76.49	0	5.2	5.7	0	0.2	0.5

RE – Section of Ramgarh village where 250 ppm esbiothrin rope was burnt w.e.f. 1 August 1990; where no rope was burnt.

Population – Ramgarh (C: 623), Ramgarh (E: 514) and Jadaonpur (C: 693).

An. culicifacies population from human and mixed dwellings to cattlesheds in experimental area.

The impact of esbiothrin smouldering rope was more pronounced when the indoor landing rate of mosquitoes on human baits was compared as between (i) RE and RC and (ii) RE and JC areas. The indoor average number of *An. culicifacies* females landed on human bait per night was 0.0 to 0.7 during August to March in RE area where esbiothrin-impregnated rope was allowed to smoulder as against 4.2 to 18.7 in RC area where plain rope was burnt. The difference was more evident in JC area where no rope was burnt. The indoor average number of females/bait/night was 4.7 to 19.5 in the corresponding period. Similar results were obtained when the average number/bait/night was compared against *An. annularis* and *An. subpictus* and total anophelines

Jadaonpur villages Distt. Ghaziabad (U.P.)

Indoor no. of mosquitoes landed/bait/night			Total *anophelines*			*Culex quinquefasciatus*			Total mosquitoes		
An. subpictus											
RE	RC	JC	RE	RC	JC	RE	RC	JC	RE	RC	JC
–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–
22.5	25.0	27.0	38.0	40.5	47.5	51.2	57.7	57.2	89.2	98.2	104.7
0	0.7	1.5	0	16.2	19.0	3.2	13.7	15.7	3.2	30.0	34.7
0	1.2	2.2	0	8.2	12.0	5.2	27.2	28.0	5.2	35.5	40.0
0	14.2	16.0	0	23.5	26.7	8.2	23.7	27.0	8.2	47.2	53.7
0.7	1.5	2.7	1.5	16.0	19.5	4.2	16.7	21.5	5.7	32.7	41.0
0	4.0	5.2	0	31.0	33.5	5.2	48.0	50.2	5.2	79.0	83.7
0	0.7	2.2	0	8.0	11.5	1.2	4.0	5.7	1.2	12.0	17.2
0	0.5	1.0	0	5.0	6.5	1.5	6.0	6.7	1.5	11.0	13.2
0	0.2	0.5	0	5.7	6.7	2.2	9.5	10.7	2.2	15.2	17.5

RC — Section of Ramgarh village where plain rope was burnt w.e.f. 1 September 1990; JC – Control village

(Table 1). However, 250 ppm esbiothrin-impregnated rope did not provide adequate protection against *Culex quinquefasciatus*. This confirms the earlier finding of Ansari *et al.*[2], who recommended 500 ppm dose against culicine.

The results of bloodmeal analysis showed that smoke from smouldering esbiothrin rope results in a marginal reduction in anthropophilic index in comparison with that of untreated rope. The anthropophilic index in the RE and RC areas respectively was as follows: August – 0.7, 1.2; September – 0.9, 1.4; October – 0.7, 2.5; and November – 2.5, 4.2.

The impact was more pronounced when the anthropophilic index was compared with that in JC area where no rope was allowed to smoulder. The index was 4.3, 2.2, 7.1 and 7.8 in the months of August, September, October and November re-

Table 2. Parasitological indices of Ramgarh and Jadaonpur villages Distt. Ghaziabad (U.P.)

Year/ Month	Parasitological indices														
	RE					RC					JC				
	BER	+ve	SPR	SfR	Case/ 000	BER	+ve	SPR	SfR	Case/ 000	BER	+ve	SPR	SfR	Case/ 000
May 90	1.16	0	0	0	0	–	–	–	–	–	2.45	1	5.8	5.8	1.44
Jun	2.72	1	7.1	0	1.94	–	–	–	–	–	3.17	1	4.5	4.5	1.44
Jul	5.25	0	0	0	0	–	–	–	–	–	3.60	0	0	0	0
Aug	1.36	0	0	0	0	7.7	9	18.75	2.08	14.44	7.07	4	8.1	2.04	5.77
Sep	1.55	1	12.5	0	1.94	5.13	4	12.5	3.12	6.42	3.75	4	15.3	3.84	5.77
Oct	1.94	1	10.0	0	1.94	4.33	2	7.4	0	3.21	3.6	4	16.0	12.0	5.77
Nov	1.16	1	16.6	16.6	1.94	2.72	2	11.7	11.7	3.21	3.17	4	18.18	18.18	5.77
Dec	1.55	0	0	0	0	2.24	0	0	0	0	2.02	8	57.14	42.85	11.54
Jan 91	0.97	0	0	0	0	2.24	0	0	0	0	1.44	1	10.0	10.0	1.44
Feb	0.97	0	0	0	0	2.56	0	0	0	0	1.01	0	0	0	0
Mar	1.36	0	0	0	0	2.72	0	0	0	0	1.58	1	9.0	9.09	1.44

RE – Section of Ramgarh village where 250 ppm esbiothrin rope was burnt from August 1990 to March 1991; RC – Section of Ramgarh village where plain rope was burnt from September 1990 to March 1991; JC – Control village where no rope was burnt. Population – Ramgarh (C: 623), Ramgarh (E: 514) and Jadaonpur (C: 693).

spectively. This substantiates the earlier observation of high density of *An. culicifacies* in cattlesheds in RE and RC areas where esbiothrin-impregnated and plain ropes were burnt in human and mixed dwellings. It may be possible that the species has deviated from human and mixed dwellings to cattlesheds where ropes were not in use and this behavioural change could be responsible for reduced anthropophilic index in RE area.

The anthropophilic index of RC area was also low in comparison to that in JC area. This may be due to smouldering of plain rope in human dwellings and mixed dwellings, which might have prevented the feeding on human host. A marginally high anthropophilic index in November in experimental section was expected because of interrupted supply of rope, owing to circumstances beyond our control.

Parasitological monitoring also revealed a considerable impact of esbiothrin smoke on transmission of malaria. The rope was burnt in human and mixed dwellings from August 1990 to March 1991. During this period 3 cases of *P. vivax* and one case of *P. falciparum* were observed in RE area as against 13 cases of *P. vivax* and 4 cases of *P. falciparum* in RC area where plain rope was burnt. However, the difference was not significant statistically as the P values were greater than 0.05. The difference was highly significant ($P < 0.05$) when cases of experimental section RE were compared with JC area where no rope was burnt. In this village, 9 *P. vivax* cases and 19 *P. falciparum* cases were found during the same period. The cases per thousand population were 7.7 in RE area and 20.8 and 60.6 in RC and JC areas respectively (Table 2). Similarly, SfR of RE area was significantly different ($P < 0.05$) when compared with that of JC areas.

Iron mesh cylinder also proved quite effective in preventing fire hazards as no incidence of fire was observed during the study period. It was also socially accepted by the inhabitants and found quite useful for hanging the rope.

The study demonstrated the feasibility of using impregnated rope to achieve epidemiological impact at least against zoophilic vector species by diversion to cattlesheds. The cost of the rope is 45 paise per metre excluding impregnation cost. The total consumption of the rope is 1.2 m/night if burnt uninterrupted from dusk to dawn. The impregnation cost is only 20 paise/m including the cost of kerosene. The total cost would be 75 paise per room/verandah as against Rs. 1.50 to 2.0 with coil and mats marketed in India. If Mylol, citronella oil or Odomos cream is used, the cost will still be high as these provide protection to individuals and remain effective only for 3-6 h. Besides the cost, most of the oil, cream or mats do not provide the same degree of protection against different species of mosquitoes[4]. In addition, most of matting devices require uninterrupted supply of electricity, which is not available in remote rural and forest areas. The present method of protection does not require any electricity skill and can also be used outdoors where inhabitants are engaged in various types of

routine activities. Though no adverse effects of smoke were observed during the study period, yet certain inhabitants did complain of the pungent smell of smoke. Health studies are therefore indicated to see if there are any adverse effects on inhabitants continuously exposed to smoke and also for extended field trial in ecologically different endemic areas to ascertain its utility in control programmes against different vector species.

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Biology of Malaria Vectors in Central Gujarat

R.M. BHATT, H.C. SRIVASTAVA and P.K. PUJARA

Biology of malaria vectors were studied in Kheda district of central Gujarat in order to understand the vector behaviour in the wake of ecological changes. *Anopheles culicifacies* and *An. stephensi* were mainly endophilic whereas *An. fluviatilis* exhibited marked exophily. All the three vectors were predominantly zoophilic. Peak biting activity period of *An. culicifacies* varied with season though biting continued in varying magnitudes throughout the night. *An. stephensi* and *An. fluviatilis* were arhythmic in their biting activity. Survival of all the three vectors was maximum during cold season owing to longer gonotrophic cycle which also yielded higher estimates for expected infective life. Instability of malaria in this area was probably due to low estimates of expected infective life for the three vectors. A wide range of breeding habitat preferences by *An. culicifacies* was observed whereas *An. fluviatilis* and *An. stephensi* showed restricted distribution.

Keywords: Malaria vectors, Biting rhythms, Resting behaviour, Vectorial efficiency

INTRODUCTION

The nature, bionomics and distribution of primary and secondary vectors of malaria, since their disruption by the eradication programme, are insufficiently known. During and since that period radical changes have occurred in ecosystems owing to deforestation, intensification of agriculture, irrigation projects and urbanization. In the wake of large-scale ecological changes, a proper understanding of the various aspects of the bionomics of malaria vectors is necessary in devising appropriate strategies for vector control[1]. There is

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great need to study the changes in vector behaviour, patterns of distribution, seasonal prevalence, vectorial efficiency and their correlation with various epidemiological situations.

There is insufficient ecological information from Gujarat on the malaria transmission dynamics and it is desirable to understand how control measures might have influenced them. A few entomological reports have appeared from this region[2-4]. Some of the bioecological aspects of the three malaria vectors, viz. *An. culicifacies, An. fluviatilis* and *An. stephensi*, and their role in malaria transmission in Kheda district, central Gujarat, are discussed in this paper.

MATERIALS AND METHODS

Studies were carried out in eight villages of Kheda district for three years from January 1989 to December 1991. The villages were representative of various biogeographic areas having considerable biological diversity and ecological habitats, leading to formation of independent ecosystems, viz. hills and foothills (1) and open terrains (7). The latter was further sub-divided into canal irrigated (2), non-canal irrigated (2), riverine (2) and coastal (1) areas. Characteristics of each area and climate have been described earlier[3, 5].

Mosquito collections

Resting mosquitoes were collected at fortnightly intervals in all the villages from indoor as well as outdoor shelters. Indoor resting mosquitoes were sampled using mouth aspirators for 15 min/shelter in the early morning hours. Four houses and four cattlesheds were sampled in each village. Indoor sampling was followed by the outdoor resting collection from suitable natural outdoor habitats and specially dug artificial pit shelters[4]. Biting collections using bovine bait trap technique were made for one year from January to December 1989 in six villages only (2 each in canal, non-canal and riverine areas). All-night collections from 1800 to 0600 hrs were made using bullock as bait in each village once in a month. Villages representing coastal and hilly areas were not sampled owing to infeasibility.

Larval collections

Immature stages of mosquitoes were collected at fortnightly intervals from all breeding sites located in and around each village using an enamel dipper. Variability in breeding habitats due to sporadic irrigation, seasonality and rainfall made collections at fixed stations impractical. Immature specimens were brought to the laboratory and reared to adults for identification.

Processing

Adult mosquitoes collected from indoor and outdoor sites were brought to the laboratory and identified. Vectors were further classified as per trophic status and were dissected to determine reproductive condition and physiological age. Trophic status was scored as unfed,

fully fed, half gravid and gravid. Reproductive age was determined by counting the number of ovarian dilatations, and parity in unfed was confirmed by observing tracheolar skins[6]. Bovine bait trap collections were identified on the same night and only vectors were dissected. Owing to insignificant differences observed in biting rhythms of malaria vectors between different physiographic areas, the monthly data were pooled and rhythm was plotted for different seasons as per cent transform of the Williams mean (Mw)[7]. Females collected from different resting sites with fresh and partially digested bloodmeals were smeared on filter paper and tested against the human and bovine antisera using agar-gel diffusion technique[8]. Survivorship (S) was estimated as $S = P^{1/gc}$, where P is the proportion parous and gc is the duration of gonotrophic cycle in days[9]. Considering survivorship to be constant with age, mean life expectancy (E) was estimated as $E = (-\log_e S)^{-1}$. The duration of sporogony (n) was calculated as a function of temperature[10] using the formula $n = T/(t\text{-}t_{min})$, where T is the total 'degree days' required for the sporogony; t is the actual average temperature in °C; and t_{min} is threshold temperature required for the parasite development. Expected infective life (EIL) was estimated using the formula of Macdonald[11] as $EIL = S^n/-\log_e S$.

RESULTS AND DISCUSSION

Resting behaviour

The distribution of the three malaria vectors among five physiographic areas and their abundance in different resting habitats are summarized in Table 1. The temporal distribution of *An. culicifacies* and *An. stephensi* has been described from this area[5] earlier, hence data on spatial distribution only are presented here. Because differences in mean abundance between species varied over time and space, comparison among areas was done separately. A one-way ANOVA and Tukey multiple comparison test ($p=0.05$) were used to compare densities in different areas after transforming monthly abundance data to $\log_e (y + 1)$. Spatial abundance of *An. culicifacies* in indoor resting collections varied significantly ($F=18.31$; $df=4, 55$; $p<0.001$) and the density was maximum (37.89 MHD) in the riverine area followed by hilly (19.14) and canal-irrigated (18.25) areas. Though *An. culicifacies* density in natural outdoor shelters did not vary significantly between the areas ($F=2.35$; $df=4,55$; $p>0.05$), its abundance in artificial pit shelters indicated significant differences among areas ($F=4.29$; $df=4,55$; $p<0.001$). *An. fluviatilis* was most abundant in hilly area ($F=7.86$; $df=3,24$; $p<0.01$) and was not recorded from non-canal area though mean abundance indicated insignificant differences between canal, coastal and riverine areas (Tukey test, $p>0.05$). This species preferred to rest in natural outdoor shelters ($F=3.67$; $df=3,28$; $p<0.01$) and pit shelters ($F=10.33$; $df=2,33$; $p<0.001$). However, it was not recorded from pit shelters in the coastal area. *An. stephensi* was abundant in low numbers in all the study villages and it showed preference for indoor shelters over the outdoor. Moreover, insignificant differences observed in its abundance among different areas

Table 1. Distribution and abundance of malaria vectors in different resting sites

Area	*An. culicifacies*			*An. fluviatilis*			*An. stephensi*		
	IRC*	ORC*	PSC†	IRC	ORC	PSC	IRC	ORC	PSC
Canal	18.25^{cd}	1.59^{a}	0.33^{ab}	0.03^{a}	0.07^{ac}	0.13^{a}	0.32^{a}	0.02^{a}	
Coastal	5.55^{bd}	3.78^{a}	0.15^{b}	0.02^{a}	0.02^{bc}		0.56^{a}	0.07^{a}	0.04^{a}
Hilly	19.14^{ac}	1.46^{a}	0.40^{ab}	0.57^{b}	0.73^{a}	2.48^{b}	1.36^{a}	0.12^{a}	0.08^{a}
Non-canal	1.74^{b}	1.52^{a}	0.05^{b}				0.39^{a}		
Riverine	37.89^{a}	3.77^{a}	0.61^{a}	0.01^{a}	0.07^{ac}	0.16^{a}	0.26^{a}	0.02^{a}	0.01^{a}

*Indoor (IRC) and outdoor (ORC) resting collections indicate man hour densities; †Pit shelter (PSC) collections indicate nos. per pit. Means followed by the same alphabet in a column were not significantly different using Tukey multiple comparison test ($p>0.05$).

Table 2. Variation in blood meal sources in malaria vectors according to the type of resting place

Resting site	Species	No.	Blood meal source in per cent			
			Human	Cattle	Human + Cattle	Others
Human dwelling	*An. culicifacies*	1213	0.41	93.24	0.41	5.93
	An. fluviatilis	5		100		
	An. stephensi	9		100		
Cattleshed	*An. culicifacies*	4060	0.25	95.49	0.32	3.94
	An. fluviatilis	18		100		
	An. stephensi	84		92.86	1.19	5.95
Outdoor	*An. culicifacies*	84		100		
	An. fluviatilis	125		93.60	1.60	4.80
	An. stephensi	4		100		
Total	*An. culicifacies*	5357	0.28	95.05	0.34	4.33
	An. fluviatilis	148		94.59	1.35	4.05
	An. stephensi	97		93.81	1.03	5.15

in all the three resting sites studied indicates less favourable breeding conditions. With the beginning of canal irrigation and shortfalls associated with irrigation, water management and consequent changes in the cropping pattern during the last three decades the entire command area in the district has become conducive for mosquitoes during spring as well. Though there was no insecticide spray in the study villages during the course of these investigations, outdoor resting habit of *An. culicifacies* and *An. stephensi* is suggestive of some change in their behaviour owing to repeated exposure to insecticides used in antimalaria programme. This phenomenon is substantiated by the fact that outdoor resting (pit and natural shelters) population of three vectors comprised all trophic stages (Fig. 1), and if there was no outdoor resting habit, the proportion of freshly fed and semigravid is expected to be roughly equal in indoor resting mosquitoes[2,12]. The proportion of semigravid to fully fed specimens in indoor collections was less (0.13) for *An. fluviatilis* which indicates a relatively higher degree of exophily in this species. The abdominal condition of outdoor resting population shows that the proportion of fully fed specimens was more than that of the semigravid which further suggests the outdoor resting habit of the vectors in this area.

Host feeding patterns

Out of the total blood smears of three species analysed, 95% reacted with human and bovine antisera, the remaining 5% were not further tested (Table 2). Over 90% of all *An. culicifacies*, *An. fluviatilis* and *An. stephensi* bloodmeals were from bovids. Owing to their low densities the sample size of the latter two species was very low and none was found to react exclusively with human antisera. Results for *An. culicifacies* are in agreement with previous observations made from different parts of Gujarat[13-15]. *An. fluviatilis* is generally regarded as predominantly anthropophilic species[12]; however, a much lower degree (0.027) of contact was reported from India[13] and similarly HBI for *An. stephensi* was found to be low (0.018).

Biting behaviour

A total of 41,552 anophelines were collected in 70 all-night bovine bait trap collections, and *An. culicifacies*, *An. fluviatilis* and *An. stephensi* accounted for 5.57%, 0.32% and 0.42% respectively.

An. culicifacies, exhibited marked variations in its feeding times. During winter, most biting occurred during the first quarter of the night which shifted to the second and third quarters during hot and rainy seasons respectively (Fig. 2). Biting took place in varying magnitudes from dusk to dawn throughout the year. Hourly means of different quarters (18-21, 21-24, 24-03 and 03-06) were transformed to $\log_e (y + 1)$ and compared using one-way ANOVA. Quarterly means differed significantly during the cold season only ($F=4.38$; $df=3,44$; $p<0.01$). Means for hot and rainy seasons were

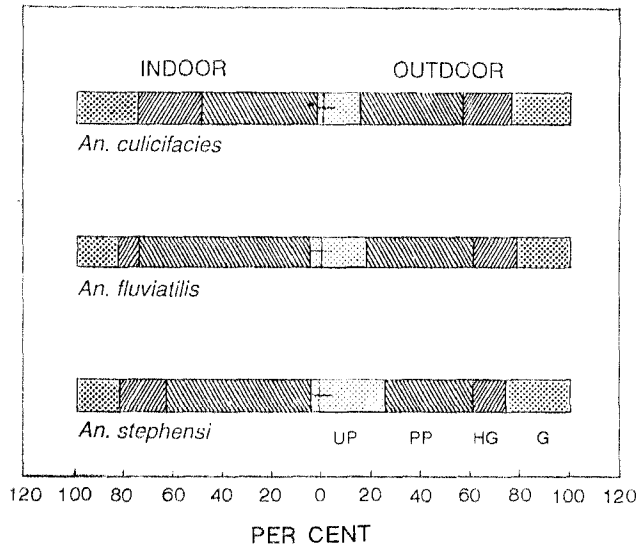

Fig. 1 : Trophic status of malaria vectors collected from indoor and outdoor resting sites

insignificant indicating less marked differences in the biting rate during these seasons though considerable variations were observed in the biting activity. A similar shift in biting activity was also observed in Punjab Province, Pakistan[16].

An. fluviatilis exhibited no definite rhythm in its feeding activity. During rainy season, 71% of the specimens were collected in the first half of the night with a peak between 2000 and 2100 hrs, which is in agreement with the previous observations made in Madhya Pradesh[17]. No significant variation in the biting activity between different quarters of the night was observed during the three seasons when tested by ANOVA.

An. stephensi was observed biting mostly before midnight, and during hot and cold seasons, maximum activity took place in the first quarter of the night. No significant shift in the biting activity was evident owing to low abundance and the means for different quarters of the night were insignificantly different when tested by one-way ANOVA. Biting continued till third and fourth quarter, though at a very low rate. These results in general agree well with those reported by Reisen and Aslamkhan[16]. However, a marked seasonal shift in its biting activity was observed.

Reproductive status

A total of 4905 *An. culicifacies*, 195 *An. fluviatilis* and 132 *An. stephensi* females, collected from different biotopes in all study villages, were dissected, and as the reproductive status in different vil-

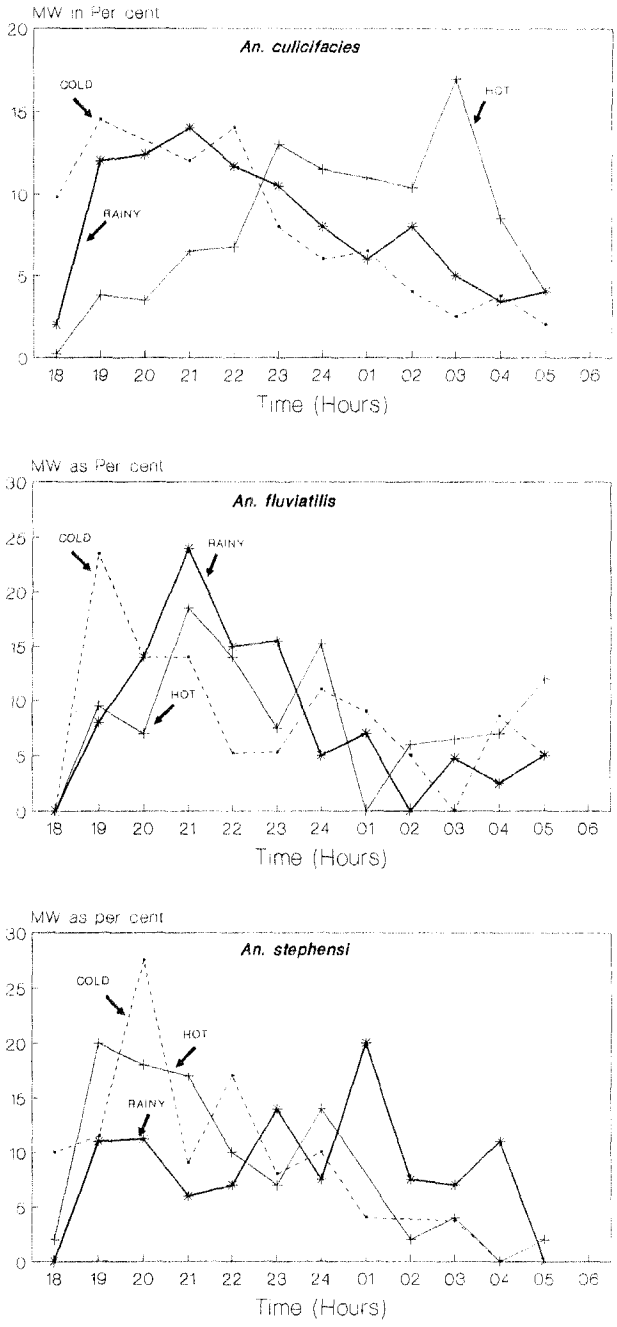

Fig. 2 : Biting rhythms of malaria vectors during different seasons

Table 3. Reproductive status, survivorship and expected infective life of malaria vectors

Species	Season*	No. dissected	Oviparity**							Survivorship†			EIL‡	
			0	1	2	3	4	5	6	P	S	E	*Pv*	*Pf*
An. culicifacies	Hot	1800	947	722	113	17		1		0.473	0.687	2.66	0.192	0.132
	Rainy	2096	1087	854	120	31	2	1	1	0.481	0.693	2.73	0.209	0.145
	Cold	1009	597	349	53	3	7			0.408	0.799	4.46	0.154	0.050
An. fluviatilis	Hot	99	56	34	6	2	1			0.434	0.658	2.39	0.127	0.083
	Rainy	7	5	2						0.285	0.533	1.59	0.019	0.010
	Cold	88	61	20	7					0.306	0.743	3.37	0.039	0.008
An. stephensi	Hot	27	15	12						0.444	0.666	2.46	0.143	0.095
	Rainy	44	23	18	2	1				0.477	0.690	2.69	0.201	0.138
	Cold	61	43	16	1		1			0.295	0.737	3.28	0.034	0.007

*Hot (Mar-Jun), Rainy (Jul-Oct), Cold (Nov-Feb); **Oviparity based on number of ovariole tubule dilatations, 0 to 6; †P = Proportion parous; S = Survival through one day ($P^{1/gc}$), where gc = 2 days for hot and rainy season and 4 days for cold season; E = Life expectancy $(-\log_e S)^{-1}$; ‡EIL = Expected infective life ($S^n/-\log_e S$), where n is the duration of sporogony estimated to be 7 and 8 days during hot and rainy season and 15 and 20 days during cold seasons for *Pv (P. vivax)* and *Pf (P. falciparum)* respectively.

lages was relatively similar the data were pooled to depict seasonal trends (Table 3). The proportion of parous *An. culicifacies* varied less between the seasons. Only 1.28% of females dissected survived to complete three or more gonotrophic cycles. There was not much difference in the survivorship. However, owing to longer gonotrophic cycle (4 days during winter as against 2 days during hot and rainy seasons), it was higher during cold season for all the three vectors, which is in agreement with the observations made in Punjab Province, Pakistan[18]. Life expectancy for all the three vector species was maximum during the cold season. Sporogony was estimated to be 7 and 8 days during hot and rainy and 15 and 20 days during cold seasons for *Pv* and *Pf* respectively. Expected infective life (EIL) of *An. culicifacies* was longer than that of *An. stephensi*, which is in agreement with observations made in Pakistan[19]. Estimates of EIL for *An. culicifacies* were comparable for the cold season with those recorded (0.20 and 0.06 for *Pv* and *Pf* respectively) by Reisen *et al*[20]. However, low EIL was estimated both for *An. culicifacies* and *An. stephensi* during hot season in our study. For *An. fluviatilis* both E and EIL were estimated to be lower than recorded in Jeypore hill area of Orissa[21]. Low estimates of EIL for all the three vectors and particularly *An. culicifacies* may be responsible for the instability of malaria in this area.

Distribution and abundance of immatures

A total of 9363 specimens of three vectors were identified out of 75,046 anophelines that emerged from 3142 collections made from different breeding habitats. For the sake of clarity and to derive a general conclusion, the data for each habitat in different areas were pooled. Spatial distribution and average abundance of three vectors are given in Fig. 3. Abundances are given as arithmetic means as well as in a logarithmic transform known as Williams Mean (M_w). The untransformed data for each species were analysed by Kruskal-Wallis (K-W) test to detect significant differences of monthly means in ranked abundance between macrohabitats. The K-W statistic, H, was compared with χ^2 to evaluate levels of statistical significance. A significant H value was interpreted as indicating a preference by a species for one or more macrohabitats. *An. culicifacies* was found in all the macrohabitats indicating its adaptability to breed in a variety of breeding habitats which in turn makes the alternative methods of malaria control through bioenvironmental methods more difficult. Comparison between habitats revealed considerable preference for river, though breeding sources associated with canal irrigation also supported more breeding than the rest of the habitats. *An. stephensi* was found to breed in eight macrohabitats and its preference for wells is supported by previous studies[3]. *An. fluviatilis* was encountered from only seven breeding habitats and comparison among habitats revealed a low level of significance. Maximum abundance was observed in freshwater seepage drain resembling narrow and shallow stream.

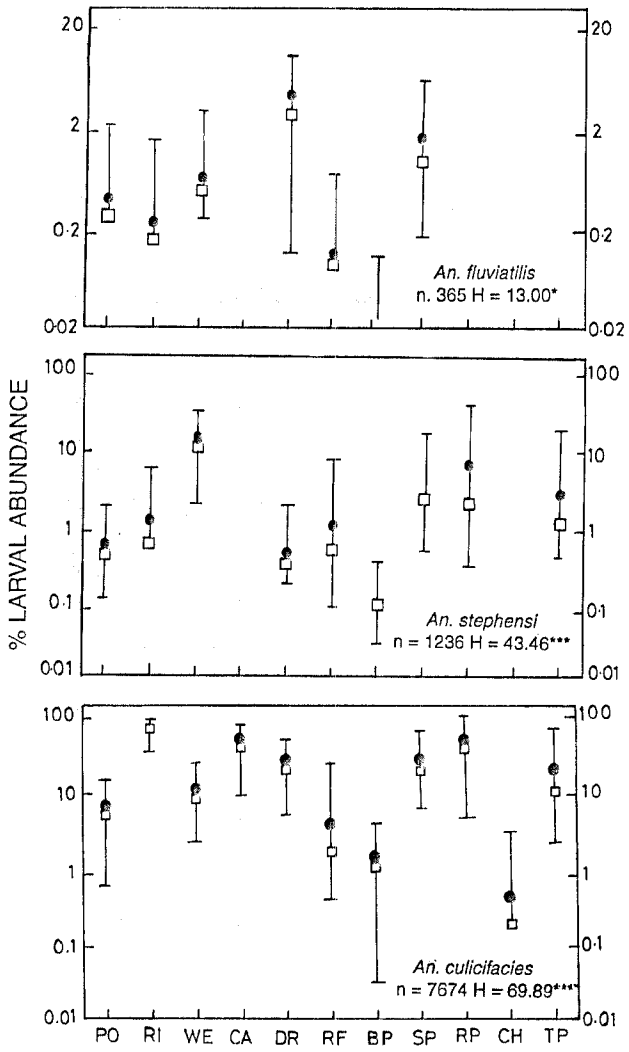

Fig. 3 : Spatial patterns of abundance of malaria vectors in different breeding habitats.

Circles represent William's mean, squares are arithmetic means bracketed by range in monthly values. Where the lower limit is not shown, it extended beneath X axis, *n* is the number of individuals identified and *H* is Kruskal-Wallis statistic, evaluated for significance by comparison with χ^2; *** $p < 0.001$; *$p < 0.05$; PO — Ponds; RI — River; WE — Wells; CA — Canal; DR — Drains; RF — Rice fields; BP — Borrow-pits; SP — Swamps; RP - River bed pools; CH — Channel; and TP — Tyre/hoof prints.

Relationship with malaria transmission

Kheda district falls under the zone of unstable malaria which is confirmed by the present findings, though perennial transmission has been reported in some riverine villages virtually free from cattle population (MRC, unpublished data), and *An. culicifacies* was incriminated as vector which is indicative of its efficiency as vector in spite of low HBI and EIL recorded in our study. With very low abundance and low HBI, *An. stephensi* seems unlikely to play any role in malaria transmission in the rural areas of Kheda district. *An. fluviatilis* was most abundant in hilly area only and had a higher HBI than the rest of the two species. Though it has been incriminated as vector from the adjoining district of Panchmahals[22], low estimates of EIL during our study call for further investigations.

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REVIEW ARTICLE

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Oxidative Stress and Malaria-Infected Erythrocytes

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This paper presents several mechanisms/pathways by which oxidative stress could cause damage to the parasites. During developmental stages of plasmodia profound alterations of the structure and function of host erythrocytes take place, in order to support the development and/or survival of the parasite. In addition an oxidant stress is also induced by the parasite. There is also an increased production of reactive oxygen species (ROS) by the parasite. This may deplete the erythrocyte of its defense mechanisms namely, superoxide dismutase (SOD), catalase, glutathione peroxidase, NADPH, NADH, glutathione (GSH) and glutathione reductase etc. Thus oxidative stress may be exerted by the growing parasite in red blood cells which are highly sensitive to such a challenge. These enhanced alterations may result in a retarded development of the parasite. Thus, the coexistence of both parasite and erythrocyte is a matter of a delicate balance. However, one cannot rule out the role of external modulations (immune pressure) inhibiting the vitality of the parasites.

Keywords: Erythrocytes, Malaria, Oxidative stress, Reactive oxygen species (ROS)

INTRODUCTION

Malaria, a protozoan/vector-borne disease, forms the best example of intracellular parasitism, which exhibits extreme biological interactions between the human erythrocytes and malarial parasite. The whole cycle of malaria is operable at

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the four sequential stages: (a) selection, (b) invasion, (c) intra-erythrocytic development, and (d) liberation of merozoites. With a total of 2.2 million people at risk, and drug resistance spreading, there is an urgent need for development of proper countermeasures. During the last decade, malaria research has been intensified both at the applied and at the basic levels. Though efforts are being directed towards vaccine development, new chemotherapeutic approaches have also been entertained. Since the pathogenicity of malaria is provoked mostly by synchronous asexual development of the parasites, this intra-erythrocytic stage of parasite development deserves a high priority of research in order to provide a new rationale for new therapeutic approaches.

The life-cycle of malarial parasite is influenced by the genetically controlled characters of the host red blood cells (RBC), surface coat of the red blood cells or the intracellular components and their metabolic pathways. These factors affect the immunity possessed by the host which may be innate/inherited or acquired[1]. Innate immunity may be attributed to inherited erythrocytic resistance provided by genetically controlled characters like abnormal haemoglobinopathies, G-6PD deficiency[2] and Duffy group antigens[3]. Acquired immunity is principally from T-lymphocyte-dependent activation of macrophages and natural killer cells which exert oxidant stress on parasitized cells. The pattern of acquired immunity to blood stage infections varies widely in different host-parasite combinations.

Asexual blood forms of malarial parasites are micro-aerophilic and sensitive to oxidant stress. The degeneration of parasites in circulating erythrocytes after exposure to oxidants is closely analogous to the "crisis forms" observed during the recovery from malaria infections as a result of cell-mediated immunity whose major effector mechanism is the imposition of oxidant stress on parasitized cells[4]. In this article we discuss the redox status of the malaria-infected erythrocytes in relation to malaria chemotherapy and host responses against the parasite. A number of articles on malaria biochemistry, chemotherapy and host-parasite relationship are available[5-9], some of which discuss the role of oxidative stress. The basic aspects of the formation and metabolism of reactive oxygen species (ROS) in biological systems are discussed elsewhere[10].

Reduction-oxidation (redox) reactions involve transfer of electrons, i.e. reducing potential increases relative to oxidizing potential. "Oxidative stress" is defined as any disturbance of the pro-oxidant-antioxidant balance in favour of pro-oxidant. Exposure of cells to conditions of oxidative stress does not necessarily result in oxidative damage to cellular macromolecules as the existing host cell defenses may be adequate or as cells upgrade these defense mechanisms[11]. When the cellular redox capacity increases, the cells may be better equipped to resist oxidative stress provided the other components of antioxidant defenses are unchanged.

The current research relating to oxida-

tive stress and malaria parasite can be summarized as follows: (a) the malaria parasite exerts oxidative stress on host erythrocytes; (b) oxidizing conditions are mimical for the growth and survival of parasite; (c) host's response to malaria by activated phagocytes; and (d) pro-oxidant drugs as effective antimalarials with potential for chemotherapeutic exploitation.

General

A number of observations have inexorably led to the conclusion that cell-mediated immunity has a direct impact on the pathogenesis of malarial parasite. Clark *et al.*[12], in a murine system, have implied an involvement of non-antibody soluble factor in host responses. Host responses to circulating intra-erythrocytic parasites involve T-cells, cytokines and phagocytes, with soluble product of phagocytes being possibly the ultimate effectors[13,14]. Ever since it became possible to culture *P. falciparum* in human erythrocytes, there is a considerable support for the idea that oxidative stress may explain reduced growth of malarial parasite in these abnormal erythrocytes[15]. The influence of dietary factors[16] may be overlooked in *P. falciparum* culture system. There may not be any direct evidence for the oxidative damage to the parasite and assessments of the redox status and antioxidant capacity of the parasite/erythrocyte may not be made. Therefore, a role of ROS in inhibition of malarial parasite growth associated with genetic traits is an interesting concept which as yet has insufficient direct supporting evidences.

Certain vitamin deficiencies have also been implicated as agents that reduce malarial parasite under experimental and clinical studies and are believed to act via ROS. Riboflavin deficiency depresses the growth of human[17-19] and animal[20] malarias *in vivo*. The explanation is the depletion of reduced glutathione (GSH) resulting from decreased activity of flavin-dependent glutathione reductase[21]. It would therefore be useful to study blood GSH and GSSG (oxidized glutathione) in malaria-infected animals or humans and to look for evidences of oxidative events[22].

In a number of studies, the effects of these oxidative agents were blocked by antioxidants or iron chelators[23,24] or there was evidence of lipid peroxidation after their administration *in vitro*[25]. It may also be noted that a number of antimalarial drugs, e.g. Primaquine[26], Qinghaosu[27,28], are also believed to be exerting their action via ROS. Thus there is a source of ROS potentially capable of damaging malarial parasites and host cells during infection. The direct evidence of killing of parasites by ROS derived from phagocytes has been with *P. yoelli*[29] and *P. falciparam*[30,31] *in vitro*. However, these studies used only a small range of antioxidant enzymes to establish a role of ROS and also the techniques for detecting the latter were not optimal. The role of oxidation in the host response to malaria does not contain any glaring inconsistencies, although there are areas where direct evidences are deficient. A number of drugs that may inhibit the activity of GSSG perox-

idase have been shown to be very effective antimalarials[32,33].

Energy status of parasite/erythrocyte unit

Owing to non-availability of stored glycogen in intraerythrocytic phase of the parasite, glucose acts as a source of energy, as it is more permeable in the parasitized erythrocyte membrane[8]. Glycolytic pathway plays an important role for glucose metabolism in different *Plasmodium* species. The enzymes in the soluble cytoplasmic portion of the parasitized cell degrade glucose ultimately to phosphoenol pyruvate (PEP).

High energy phosphate bond donated/generated is utilised for the conversion of ADP to ATP, whereas pyruvate and pyruvate kinase act as catalyser. Pyruvate is then converted to lactate by lactate dehydrogenase along with reoxidation of glycolytically formed NADH, which finally involves an anapleutic reaction involving CO_2 fixation through PEP carboxy kinase on PEP carboxylase. These enzymes would form small amounts of oxaloacetate and other Kreb's cycle intermediates used in the biosynthetic processes[34,35]. Finally it regenerates malate in cytoplasm which can cross mitochondria and serve as mitochondrion substrate. *In vitro* it has been established that addition of CO_2 and PEP favours plasmodial development/growth[36-38]. Various workers have speculated that NADP is a more effective coenzyme than NAD in malarial species. In the adult cell, during pentose pathway, NADPH is the only source for maintaining glutathione tripeptide in the reduced state which affects other enzymatic reactions and prevents the accumulation of met-haemoglobin[39].

It appears that the parasite-erythrocyte complex has the capability of carrying out some reactions of the phosphogluconate pathway and scientists have also visualised that a small proportion of glucose is utilised by parasitized red cells via pentose phosphate pathway[40]. There are still conflicting reports whether the parasite itself is capable of forming 6-phosphogluconate from G-6P, thereby generating NADPH or it relies on the host cell. During infection, NADPH remains at the same level but NAD, NADP and NADH increase from 1.5 to 2.0 times[38]. The parasite presumably depends on the host cell for the supply of NADPH[39]. Roth *et al.*[41] pointed out that the parasite-specific NADPH generating enzyme glutamate dehydrogenase is not present in normal red cells and this renders *Plasmodium* relatively immune from oxidative stress. It has been also reported that the ratios of NADPH/NADP and NADH/NAD are not concrete in *P. berghei* infection and percentage of NAD status is 10 times higher than that of NADP in normal and infected blood cells[37].

Glutathione metabolism

A major defense system for the detoxification of reactive oxygen species in the red blood cells is due to glutathione redox cycle, and enzymes essential for the cycle are glutathione peroxidase ($ROOH + 2GSH \rightarrow ROH + H_2O_2 + GSSG$) and glutathione reductase ($GSSG + NADPH + H^+ \rightarrow 2GSH + NADP$). The inhi-

bitors of these enzymes impair intra-erythrocytic growth of malarial parasites[10,42].

The importance of glutathione lies in knowing whether the loss of glutathione or inactivation of glutathione reductase is essential for the inhibition of parasite growth. Availability of GSH and its association with oxidative and reductive enzymes in the parasite/erythrocyte unit is not seemingly perturbed even when the erythrocyte parasite load is high[11]. Mohan *et al.*[43] demonstrated the extent of reduced glutathione, activity of glutathione peroxidase, amount of membrane lipid peroxidation products and the extent of hemoglobin release from host erythrocytes during *in vitro P. falciparum* growth. They examined the extent of host-cell membrane peroxidation due to parasite-derived ROS in the absence of any immune effector cells and also reported the degree of *in vitro* hemolysis as well as the status of the glutathione antioxidant system at different stages of parasite growth.

With increased parasite load of *P. vivax*, complex changes occur in the antioxidant defence mechanism of the parasitized red blood cells (PRBCs) particularly in glutathione cascade enzymes[22]. But following treatment with chloroquine the enzymes are restored to the levels of control and nonparasitized RBCs.

Ascorbate vitamin and membrane lipids

Trager[44] commented that relatively few cofactors are essential for the intracellular development of erythrocytic stages of malarial parasites. The pentothenate is not used directly by the parasites but rather coenzyme A synthesised by the host erythrocyte is utilised. Vitamin E acts as a major chain-breaking antioxidant of lipid phase and its concentrations might be related to oxidizable lipid[45]. Researchers reported that the parasitized blood cells took up dehydroascorbate more rapidly than in normal erythrocytes and gained the ability to take up reduced ascorbate which is a sensitive indicator of oxidative stress[46].

Lipid reserves are absent in the erythrocyte stages of malarial parasites. Therefore, malarial parasites are incapable of fabricating fatty acids and cholesterol *de novo*. *Plasmodium* for its lipid requirements thus relies on dynamic exchanges with blood plasma[8,47]. Mohan *et al.*[48] reported high plasma malonyldialdehyde (MDA) concentration (an index of lipid peroxidation) in *P. falciparum* patients as compared to controls, which provides evidence for the changes in the antioxidant status of the blood. Further, *in vitro* studies confirmed that the lipid peroxidation takes place when parasitized erythrocytes are exposed to oxidants.

Susceptibility of erythrocytes to oxidant stress

The principal manifestations of oxidant stress on erythrocytes are reduction in their content of reduced glutathione, lipid peroxidation, hemoglobin (Hb)-denaturation and formation of inclusions known as Heinz bodies[49]. Erythrocytes

damaged by oxidants but not lysed are rapidly removed from the circulation by the spleen and liver. Erythrocytes deficient in G-6PD are more sensitive to oxidative stress than those with a normal content of this enzyme. The activity of these protective enzymes (SOD, G-6PD etc.) declines with erythrocytic age. However, the sensitivity to oxidant stress increases[50].

Agents known to produce oxidant damage in erythrocytes include phenylhydrazine and alloxan, which forms a redox system with dialuric acid[4]. The production of O^- ions and their interaction with a hydrogen atom transferred from the organic molecule, could generate powerful oxidant radicals[51]. Baehner *et al.*[52] incubated G-6PD-deficient erythrocytes with normal human leukocytes in which respiratory burst had been triggered by phagocytosis. Hence, activation of a respiratory burst in leukocytes with concomitant production of O_2^- and H_2O_2 can impose oxidant stress on erythrocytes.

This biochemical mechanism of oxidant stress on the red cells is relevant to provide an explanation for the mechanism why Hb-S and G-6PD deficiency confers resistance to malaria and by which cell-mediated immune response may be protective[1, 53].

Factors that increase and decrease susceptibility of erythrocytes to oxidant stress

Four components of erythrocytes function in concert to counteract oxidant stress: the erythrocyte membrane, hemoglobin (Hb), protective enzymes and antioxidants such as vitamin E and vitamin C. The normal adult Hb (HbA) carries out its oxygen transport and defects in Hb or protective enzymes and antioxidants such as vitamin C and E can increase the sensitivity of erythrocytes to oxidants[1]. The oxidised Hb bound to the membrane initiates lipid peroxidation and deoxy Hb (Fe^{2+}) is protonated and liganded with anions. The mechanism of direct electron transfer from reductant to bound O_2, which can interact with many biological molecules, is of special importance in the interaction with redox-active metal ions[4].

The changes in Fe coordination converts heme Fe into a powerful oxidant species and can increase its capacity to initiate lipid peroxidation, which is associated with the formation of malonyldialdehyde, which can cross-link proteins and other biological molecules[54]. This results in the autoxidation of polyunsaturated fatty acids releasing free radicals, which in turn damage some of the biological systems[43,48]. Some lipid hydroperoxides, e.g. those produced by the lipoxygenase and cyclo-oxygenase systems, have potent pharmacological activity and may regulate biochemical pathways in parasites as they do in other cell types. Exogenases, hydroperoxides such as *tert*butyl hydroperoxide, can impose oxidant stress on cells by consuming reduced glutathione through glutathione peroxidase and thereby oxidising NAD (P) H[4]. Human erythrocytes deficient in GSH peroxidase are very sensitive to oxidant drugs and G-6PD-deficient red cells have effects on the dietary con-

stituents, e.g> fava beans *(Vicia feba)*, a metabolite, exerts oxidant stress on erythrocytes[55].

Production of superoxide and hydrogen peroxide by leucocytes

The stimulated phagocyte cells result in respiratory burst, glucose oxidation and oxygen consumption and generate NADPH, the oxidation of which is linked to the reduction of O_2 through a series of enzymes, including a flavo protein in the plasma membrane[56]. The enzyme accepting electrons from NADPH oxidises and catalyses one electron reduction of O_2 with the formation of the superoxide anion radical and O^- released by phagocytosis can be detected by reduction of nitro blue tetrazolium, the reduction being inhibited by superoxide dismutase. The dismutation can take place spontaneously, but the reaction is accelerated in the presence of the enzyme superoxide dismutase[57].

Oxidant stress exerted on erythrocytes by malarial parasites

Etkin and Eaton[40] argued that G-6PD-deficient cells are less capable of the development of malarial parasites than in G-6PD normal cells and the cardinal characteristic of G-6PD red cell is a marked sensitivity to oxidant stress, and malaria parasite might itself generate oxidants within the infected cells. *P. falciparum*, like *P. berghei*, probably acquires most of its superoxide dismutase (SOD) from its host, since parasite-associated SOD is predominantly cyanide-sensitive and has the same p^I as host SOD. Unlike *P. berghei*, however, late stages of *P. falciparum* contain an additional SOD isozyme which is not cyanide-sensitive and may represent an endogenous enzyme[58]. Endogenous SOD has not been found to be present in *P. vivax* and therefore it adopts SOD from the host cell erythrocytes[59]. It has also been visualized that *P. berghei*-infected mouse red cells accumulate met-Hb *in vivo* and have increased sensitivity to oxidants *in vitro* and catalase within infected red cells is readily inactivated by 3-amino-1,2,4-triazole[40]. In addition to functioning as the active site of enzymes and O_2 combining site of Hb and myoglobin heme is known to have major regulatory effects including activation of a factor (eIF 2α) required for the initiation of protein synthesis. Thus, it could participate in the regulation of metabolism in *Plasmodium* species, and chloroquine is thought to function as a heme chelator[4].

Conceivably the primary metabolic effect of oxidants is on the host erythrocytes with secondary effects on the parasite, e.g. oxidant drugs produce loss of K^+ from erythrocytes and this could reduce their capacity to support parasite multiplication[4]. Recently Mohan *et al.*[48] supported the earlier view of Allison and Eugui[4] that the more mature the parasite the more vulnerable the destruction. These observations further emphasize the need for an early treatment of *P. falciparum* malaria to reduce the extent of haemolysis and life-threatening anaemia, particularly in children of malaria-endemic areas.

Effects of oxidants on malaria parasites in erythrocytes

Morgan *et al.*[60] found NADPH-oxidase system unstable in leukocytes, so they use another oxidant enzyme, polyamine oxidase, which activates macrophages and in the presence of substrate reacts with molecular oxygen to generate H_2O_2, a well-known oxidant[23]. Therefore, the degeneration of parasites in circulating erythrocytes after exposure to oxidants is closely analogous to the crisis forms observed during recovery from malarial infection as a result of cell-mediated immunity. A major effector mechanism of cell-mediated immunity is the imposition of oxidant stress on parasitized cells[61].

Clark and Hunt[23] reported that injection of alloxan produced intracellular erythrocytic degeneration of *P. vinckei* in mice. Their conclusion of the production of superoxide by alloxan-dialuric acid redox system and by iron-catalysed hydroxy radical generation is supported by the observation that superoxide dismutase and catalase protected erythrocytes against hemolysis. Clark and Hunt[23] also support the view that superoxide is involved in immunity.

This raises the possibility of a role of O_2^- in erythrocyte damage and immunity. Any direct reaction of aqueous superoxide with the cellular substances that may account for the toxicity of oxygen and role of hydroxyl radicals *in vivo* is questionable. However, other reactions such as conversion of redox-active metals into powerful oxidants, generation of lipid peroxides, hydrazine, and phenyl hydrazine to reactive intermediates are likely to be more important in biological effects. It might be possible that poor delivery of experimental antioxidants to erythrocyte membranes *in vivo* may be the cause of many inconsistencies. It is also possible that mouse erythrocytes could be loaded with antioxidants *in vitro* to provide cells well protected against oxidation as potential host of parasites. Similarly provision of red cells enriched with antioxidant enzymes could be achieved by using donor cells from transgenic mice.

Overall, a role of oxidant stress in the host response to malaria is certainly consistent with the published data. However, there are a number of weak links in the chain of evidences and we have identified several of these. The need for further understanding of the mechanisms by which oxidants suppress malaria is not simply academic. The elaboration and application of methods for the protection against malaria require a more detailed understanding of the ways in which malaria is exacerbated by a gravid state. Moreover, as the prospects of vaccine against malaria grow, the need to recognise other immunological manifestations that cause harm to the host and initiate pathogenesis has to be documented.

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SHORT NOTE

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A Note on *Anopheles culicifacies* Sibling Species Composition in Stone Quarry Belt of District Allahabad (U.P.)

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Keywords: *Anopheles culicifacies*, Sibling species, Stone quarries

Biological species which are morphologically cryptic but genetically acryptic are termed as 'sibling species'. These have been found to vary in their vectorial capacity, ecology and behaviour[1], and hence the importance of identification of sibling species in taxonomically identified mosquito vectors. Such sibling species complexes have been discovered in several *Anopheles* mosquitoes. *An. culicifacies*, a well-known rural malaria vector in the Indian subcontinent, has been identified as a complex of four sibling species A, B, C and D with a specific distribution pattern[2]. Further, evidences suggest that primarily species A and C, and to some extent species D also, are implicited in the transmission of malaria[3], while the role of species B in malaria transmission appears to be doubtful[4]. It is, therefore, extremely important to map all areas under the influence of *An. culicifacies* up to the sibling species level for a better understanding of malaria transmission dynamics so as to formulate an appropriate vector control strategy. In this note we report the sibling species composition of *An. culicifacies* from Shankargarh block of district Allahabad, which is a

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famous stone quarry area and highly endemic for malaria with preponderance of *P. falciparum* malaria[5].

Seven collections of indoor resting mosquitoes were made between January 1988 and May 1989 from 12 high malaria incidence villages, viz. Bashera, Akoria, Bhagdeva, Garraiah, Gobra Hibar, Paguar, Kalyanpur, Kasota, Atrisam-daria, Mavaiyakalan, Surval Chandel and Pure Baghel of PHC Shankargarh. All collections by suction tube method were done between 0600 and 0800 hrs both from human dwellings and cattlesheds. Ovaries of all semigravid *An. culicifacies* females were pulled and fixed in modified Carnoy's fixative (glacial acetic acid:methanol :: 1:3). Polytene chromosomes were prepared following Green and Hunt[6], and sibling species were identified following the diagnostic inversion karyotypes[2].

A total of 14 anopheline species were collected from study villages, viz. *An. culicifacies, An. annularis, An. subpictus, An. stephensi, An. pallidus, An. nigerrimus, An. splendidus, An. barbirostris, An. fluviatilis, An. tessellatus, An. vagus, An. aconitus, An. varuna* and *An. turkhudi. An. subpictus,* followed by *An. culicifacies and An. annularis,* was the predominant species whereas *An. turkhudi* was the rarest species.

The composition of the sibling species of *An. culicifacies* is given in Table 1. Three sibling species of *An. culicifacies* A, B and C were found sympatric in the Shankargarh PHC villages with the cumulative per cent composition of 64, 25 and 11 respectively (Fig. 1). In April 1988 and January 1989 collections, both inversion heterozygotes and homozygotes were found. Since the i^1 inversion which is polymorphic in species A is also diagnostic for species D[7], the data for these two months were analysed and the populations were found to be in Hardy Weinberg equilibrium with nonsignificant chi-square values. This suggests random mating between the alternative forms, and i^1 inversion is polymorphic in species A population from this area. Species A was found to be predominant in all months of collection whereas species B was maximum in the month of August, which is an active rainy month in Shankargarh area. In the dry months, species A constituted 100% of the collection. The results are, by and large, in conformity with earlier observations[8].

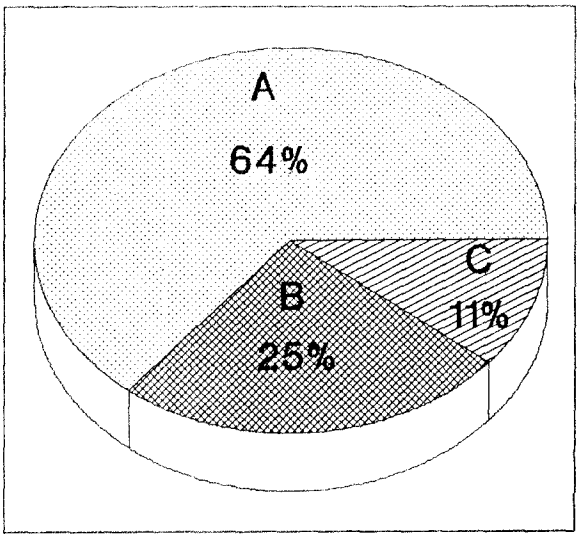

Fig. 1 : Proportion of *An. culicifacies* sibling species A, B and C in quarry area of Allahabad district (U.P.)

Table 1. Sibling species composition of *An. culicifacies* in stone quarry area of District Allahabad (U.P.)

Month/ Year of collection	Total no. of specimens estimated	Sibling species composition					
		Species A				Species B	Species C
		A	A^1	A/A^1	Total		
Jan 88	53	21	–	–	21 (39.6)	20 (37.7)	12 (22.6)
Feb	16	12	–	–	12 (75)	2 (12.5)	2 (12.5)
Mar	22	22	–	–	22 (100)	– (0)	– (0)
Apr*	21	2	12	7	21 (100)	– (0)	– (0)
Aug	11	–	3	1	4 (36.4)	6 (54.5)	1 (9.1)
Jan* 89	26	1	13	2	16 (61.5)	9 (34.6)	1 (3.8)
May	9	1	4	–	5 (55.6)	2 (33.3)	1 (11.1)
Total	158	59 (37.3)	32 (20.3)	10 (6.3)	101 (63.9)	40 (25.3)	17 (10.8)

*Subjected to chi-square test; χ^2 values for April 1988 — 0.58 (n.s.), January 1989 — 2.93 (n.s.). Figures in parentheses are percentage composition.

There appears to be a direct relationship, besides other factors, between high malaria incidence in and surrounding villages included in our study and the presence of a higher proportion of vector sibling species A and C of *An. culicifacies* throughout the collection period in these villages. Hence, we suggest that detailed investigations on the seasonal distribution and breeding sites preference of sibling species complex of *An. culicifacies* be undertaken to gain a better understanding of the epidemiology and transmission dynamics of malaria in stone quarry zone of district Allahabad.

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LETTER TO THE EDITOR

Indian Journal of Malariology
Vol. 31, June 1994, pp. 92-93.

High Level Chloroquine Resistance of *Plasmodium falciparum* in Madras, Tamil Nadu

Sir — Strains of *Plasmodium falciparum* resistant to chloroquine have been recently recorded in Madras[1]. Laboratory studies have confirmed low-level *in vitro* resistance, with minimum inhibitory concentration (MIC) of chloroquine of 8 or 16 p-mol[1]. Here we report high-level drug resistance of *P. falciparum* in a subject who was infected in Madras. A 25-year-old man, employed as a painter at Madras, developed high spiking fever from 18 October 1993. Two days later the illness was diagnosed as malaria and treatment was given. Since he did not improve, he came to CMC, Vellore on 23 October. His blood smear showed ring forms of *P. falciparum* with a parasitaemia of 2.4%. After collecting urine for chloroquine screening, and blood for testing drug sensitivity of the parasite, he was treated with quinine and doxycycline. On Day 3 his blood smears showed no ring forms of the parasite.

The Wilson-Edeson test[2], used to screen for chloroquine in the urine, was positive, showing that the patient had actually taken chloroquine prior to coming here.

In vitro drug sensitivity was measured by the method of Reickmann *et al.*[3], using complete RPMI-1640 medium. An MIC value of 8 p-mol or more of chloroquine base indicates resistance. There was no inhibition of maturation of Plasmodium even at 64 p-mol level of chloroquine.

His chloroquine therapy had consisted of 9 tablets over a period of 24 h. This is equivalent to 1350 mg of chloroquine base, or about 25 mg/kg. This is adequate for treating chloroquine sensitive infection and also for the *in vivo* test for chloroquine sensitivity as recommended by the WHO[4]. He did not have symptomatic improvement, and asexual parasitaemia persisted. This indicates RII or RIII level of

resistance. In the *in vitro* test, 64 p-mol of chloroquine failed to inhibit maturation of the parasite. The true MIC might have been even higher since the inoculated blood from the patient would have contained residual chloroquine.

Since the patient had not travelled outside Madras for over a month prior to his illness, he must have acquired the infection at Madras.

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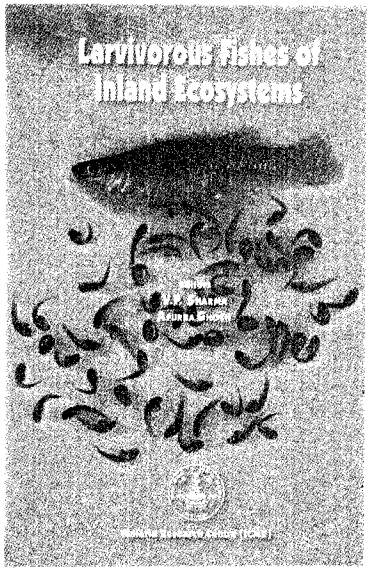

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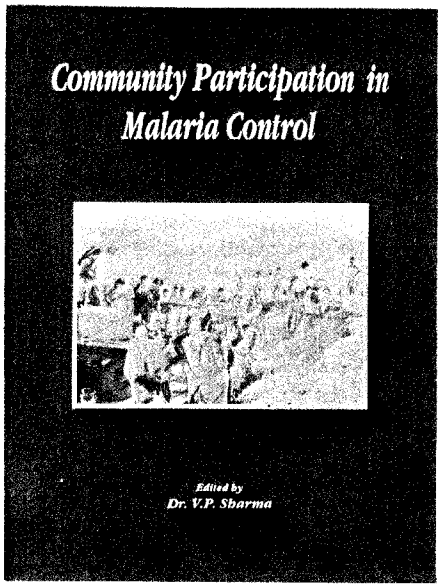

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