ENTOMOLOGY

Vector Biology

Anopheles culicifacies Complex

Bionomics and Distribution Pattern of Members

Cytological examination of *Anopheles culicifacies* samples collected from Tumkur district of Karnataka revealed that species A and B were sympatric and a good correlation between sibling species prevalence and malaria incidence was observed. In villages having high malaria incidence species A, an established malaria vector, comprised ~90% of total *An. culicifacies* population. Similarly in District Kheda (Gujarat) species A, B and C were found to be sympatric with predominance of species A in the villages with high malaria incidence and species A was polymorphic for i^1 inversion. Examination of *An. culicifacies* samples from Chhindwara district of Madhya Pradesh showed prevalence of species B, C and D in the study villages with predominance of species C. In Districts Sundergarh and Keonjhar (Orissa) species B and C were found to be sympatric and almost in equal proportion. Blood meal source analysis and vector incrimination studies revealed these species to be zoophagic and playing a secondary role to *An. fluviatilis* species S in both the districts.

Molecular Diagnostic Assays for the Identification of Members of *An. culicifacies* Complex

In continuation of the earlier work on diagnostic assays for the identification of members of *An. culicifacies* complex, a species-specific diagnostic method was developed by designing primers from D3 domain of 28S ribosomal RNA. The amplified product was sequenced directly. Alignment of sequences revealed similarity of sequence between species A and D, and between B, C and E. A multiplex PCR was standardized using two universal primers for D3 region and two allele specific primers, which can differentiate species A/D from species B/C/E (Fig. 1).

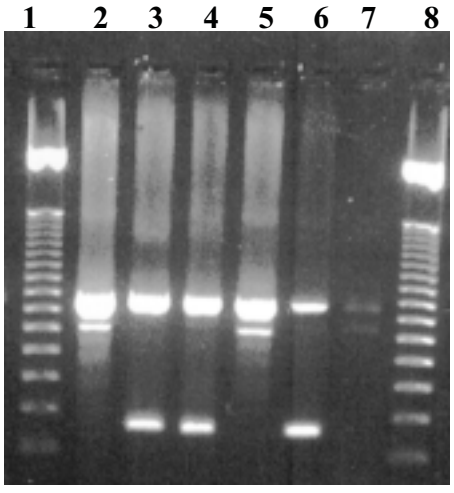


Fig. 1: PCR products obtained by the primers designed from D3 region of 28S rRNA electophoresed on 2% agarose gel. Lane 1 & 8 : 50 bp marker; Lane 2 : *An. culicifacies* sp. A; Lane 3 : *An. culicifacies* sp. B; Lane 4 : *An. culicifacies* sp. C; Lane 5 : *An. culicifacies* sp. D and Lane 6 : *An. culicifacies* sp. E from Rameshwaram

The diagnostic assay is being tested on cytologically identified field samples. Similar results were obtained by *Alu* I digestion of COII and COI amplicons and by *Rsa* I digestion of ITS2 amplicon. Field samples from Districts Jabalpur, Kheda and Hardwar, pre-identified cytogenetically, have been analyzed using COII/RFLP and ITS2/RFLP. Perfect correlation has been found as per identification of species A and B using these two DNA techniques and cytogenetic identification.

Development of Microsatellite Markers for *An. culicifacies* Species A (WHO/ TDR Funded Project in Collaboration with Yale University, USA)

A genomic library of *An. culicifacies* species A was constructed and was screened with ^{32}P labelled GT_{15} and GA_{15} probes. Fifty-four positive colonies were picked up and sequenced, and 12 microsatellite markers were obtained from the analysis of the sequences. For each marker primers were designed from unique flanking sequences. The sizes of these markers ranged between 110 and 170 bp. At present we have a total of 17 markers, 5 developed at the Yale University and 12 at MRC. Five markers were tested for polymorphism in two laboratory colonies of species A established from field collected *An. culicifacies* colonies from villages Burari (Delhi state) and Dehra (U.P.), and one each of species B (Ladpura, Haryana) and species C (Jabalpur, M.P.). All these markers were found to be polymorphic.

Molecular Cloning and Expression of Pro-phenoloxidase Gene from *An. culicifacies* Refractory to *P. vivax* (Collaboration with ICGEB, New Delhi)

The refractory mechanism in *Plasmodium vivax* refractory to *An. culicifacies* mosquito is expressed as melanotic encapsulation of early stage of malaria parasite in the midgut. The melanotic encapsulation in insects is the result of phenoloxidase cascade. To characterize the pro-phenoloxidase (proPO) gene from this refractory strain, total RNA was extracted and first strand cDNA was synthesized using RT-PCR. Using degenerate primers based on conserved amino acid sequences of proPO, a 700 bp PCR product was amplified and sequenced after cloning in pGEM-T vector. The gene specific primers were designed and full gene sequence was obtained using 5' and 3' RACE. Based on full sequence, the 5' and 3' end primers were designed and full length cDNA comprising of 2.7 kb fragment was isolated. The 2.4 kb fragment of this proPO was cloned in pET32, an expression vector and the expression was studied in host strain of *E. coli*.

Molecular Cloning of Serine Protease from *An. culicifacies* Refractory to *P. vivax:* The gene encoding serine protease has been cloned from susceptible and refractory strains. Analysis of sequence from these clones did not reveal any difference between the two strains of mosquitoes. However, activity gel staining from the serine protease revealed its presence only in the refractory strain and no activity was observed

in the susceptible strain. Further investigation on molecular mechanism of refractoriness is in progress.

Molecular Characterization of Chitinase: A 1.6 kb gene coding for chitinase of *An. culicifacies* has been cloned and expressed in *E. coli*. The chitinase is synthesized as a zymogene and activated upon cleavage of the pro-region. The role of pro-region peptide on the enzymatic activity of chitinase is being evaluated.

Anopheles fluviatilis Complex

Distribution, Bionomics and Biology of Sibling Species

Three surveys during summer, monsoon and post-monsoon seasons were carried out to study the dynamics of species S, T and U populations in Keonjhar, a highly malarious district of Orissa state contributing maximum number of deaths due to malaria. *An. fluviatilis* females collected from villages in PHCs Telkoi, Bhagamunda and Banspal were analysed for sibling species composition, their host preference and vectorial potential.

Cytological examination of the samples revealed that species S and U are prevalent in the district with predominance of species S in all the seasons. Species S was found to be highly anthropophagic with human blood index (HBI) ranging from 0.97 to 0.99. Cytologically identified specimens were subjected to enzyme-linked immunosorbent assay (ELISA) for vector incrimination. A good number of specimens belonging to species S were found positive for *P. falciparum* and *P. vivax* circum-sporozoite (CS) antigens and the overall sporozoite rate was 4.21%. These observations strongly suggest that *An. fluviatilis* species S is the principal vector of malaria in District Keonjhar. Surveys carried out in District Tumkur (Karnataka) revealed that only species T is prevalent in the villages surveyed and this species was found to be polymorphic for q^1 inversion. Efforts are being made to establish cyclic colonies of species S, T and U in order to study post-mating barriers among them and their phylogenetic relationship. In this regard short-term culture of *An. fluviatilis* species S was established and a cross was made between species S females and species T males. Per cent egg hatchability was around 80 and the hybrid males and females were found with normal reproductive organs indicating that premating barriers are responsible for reproductive isolation between these two species.

Since there is distinct difference in feeding preference of the members of *An. fluviatilis* complex, a study has been initiated to examine the mouth parts of sibling species for morphological variations. Preliminary observations have shown variations in the length and number of teeth in the mandibular blade of species S and T. The work is in progress.

Molecular Assay for Differentiating Members of *An. fluviatilis* Complex (Funded under 'Genome Project' of ICMR)

An allele-specific polymerase chain reaction-based diagnostic assay was developed for the differentiation of all the three members of *An. fluviatilis* complex, species S, T and U. The assay is based on the differences in nucleotide sequences of D3-domain of 28S ribosomal RNA in different members of the complex. For development of diagnostic assay D3-domain of 28S ribosomal RNA of cytologically identified specimens of species S, T and U was amplified using universal primers and sequenced directly from both the directions. The allele-specific primers were designed based on differences in sequences. A multiplex PCR using four primers (2 universal and 2 allele-specific) was optimized which can differentiate all the three members of the complex (Fig. 2). The validation of diagnostic assay against cytologically confirmed specimens of species S, T and U collected from different parts of India with varying sympatricities is underway.

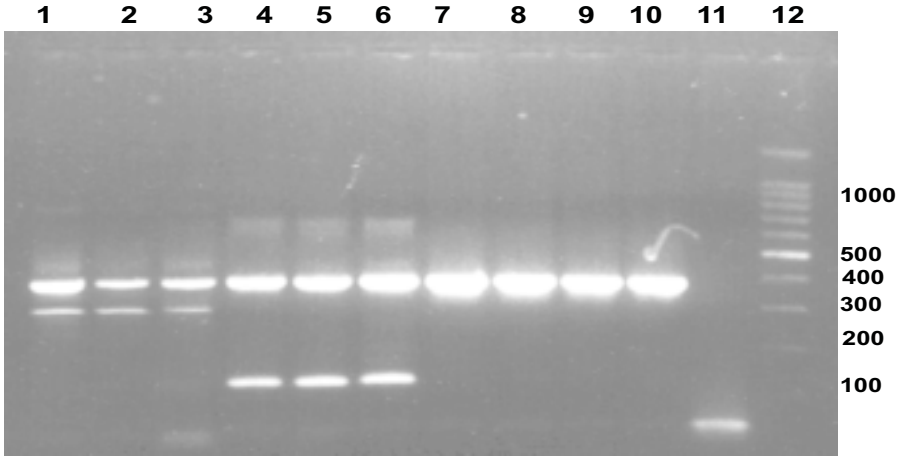


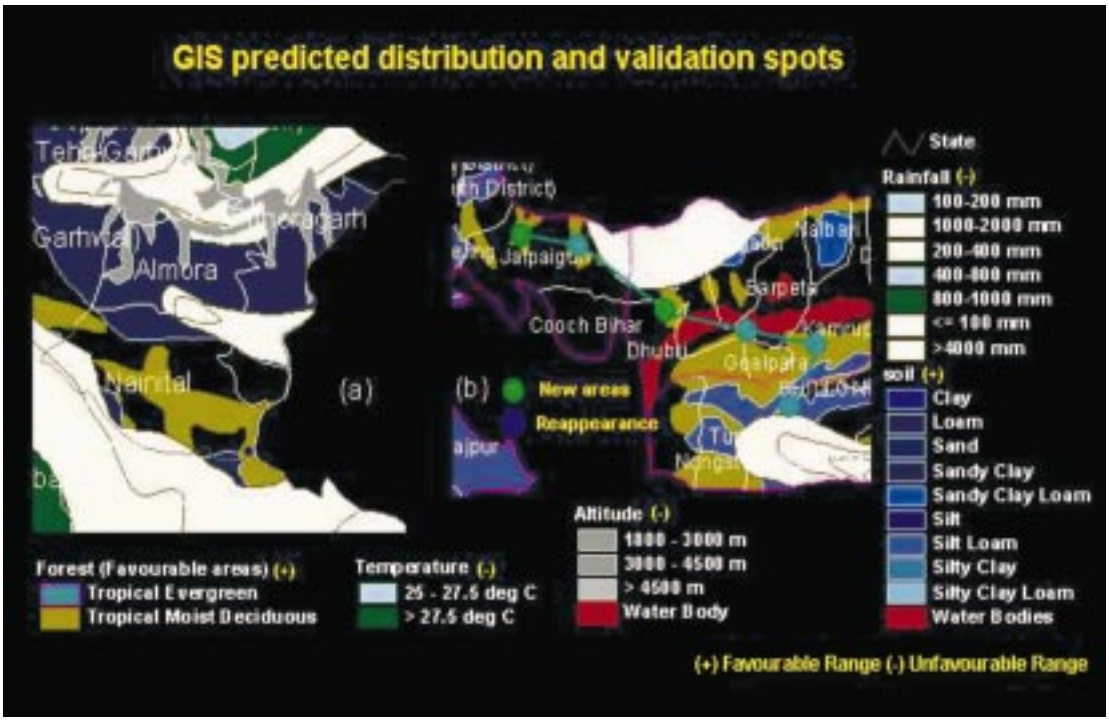
Fig. 2: Differentiation of members of *An. fluviatilis* species complex: PCR product as seen on 2% agarose gel containing ethidium bromide under UV illumination (Lanes 1–3, species S; lanes 4–6, species T; lanes 7–10, species U; lane 11, negative control, without DNA; and lane 12, 100 bp DNA ladder)

Mapping of Indian Anophelines (Funded under ICMR Task Force Project on GIS and RS)

An. minimus, the species of hill and foothill areas was mapped using GIS and compared with the reported distribution pattern. These two were found to have good

matching, there are many new areas in GIS distribution map where species is likely to be found, no survey reports are available for these areas. For validation of GIS results field surveys were conducted in : (a) species reported areas; and (b) new areas. In Nainital (Uttaranchal) surveys prior to 1951 showed presence of *An. minimus* but later the species was reported to have disappeared. GIS predicted presence of this species in Banbasa area, District Nainital and interestingly, a team visited precisely the sites predicted by GIS and collected mosquitoes from these areas (Fig. 3a). A few specimens were identified as *An. minimus*. Surveys were conducted in the months of May, July and August and the number of *An. minimus* specimens collected were 2, 12 and 16, respectively.

Precision surveys in eight locations of three other states namely West Bengal, Assam and Meghalaya were conducted in October (Fig. 3b). It is interesting to find that *An. minimus* specimen could be collected in a good number in all GIS predicted locations. Out of all these locations *An. minimus* has been reported from Dhubri for the first time. It is worth mentioning to point out that in an earlier survey in 1993 in Dhubri, *An. minimus* was not encountered. However, GIS study identified the specific locations in the same district where the species is likely to be found and during precision surveys, *An. minimus* specimen could be collected from these locations (Fig. 3b).



Figs. 3(a&b): Self validation spots in GIS predicted distribution areas of *An. minimus*. Red dots show areas where the species has been reported earlier, while pink dot show new niches.

GIS predicted distribution was mapped for *An. fluviatilis* which is an efficient vector of hills and foothill areas of the country. It is distributed at all heights from sea level up to 2500 m but most preferably from 150 to 1800 m . Temperature between 20 and 30ºC is optimum, heavy rainfall is not suitable. Sandy loam, fine sandy loam, loam, silt loam, clay loam are favourable soil types. Taking into consideration these conditions thematic mapping was done, favourable zones were integrated using GIS, the results were compared with the reported distribution and found to be correct. Distribution of *An. minimus* and *An. fluviatilis* is overlapping in certain areas, information collected during validation of distribution of *An. minimus* in Banbasa, Distt. Nainital, Uttaranchal showed the presence of *An. fluviatilis* in these areas (Fig. 4).

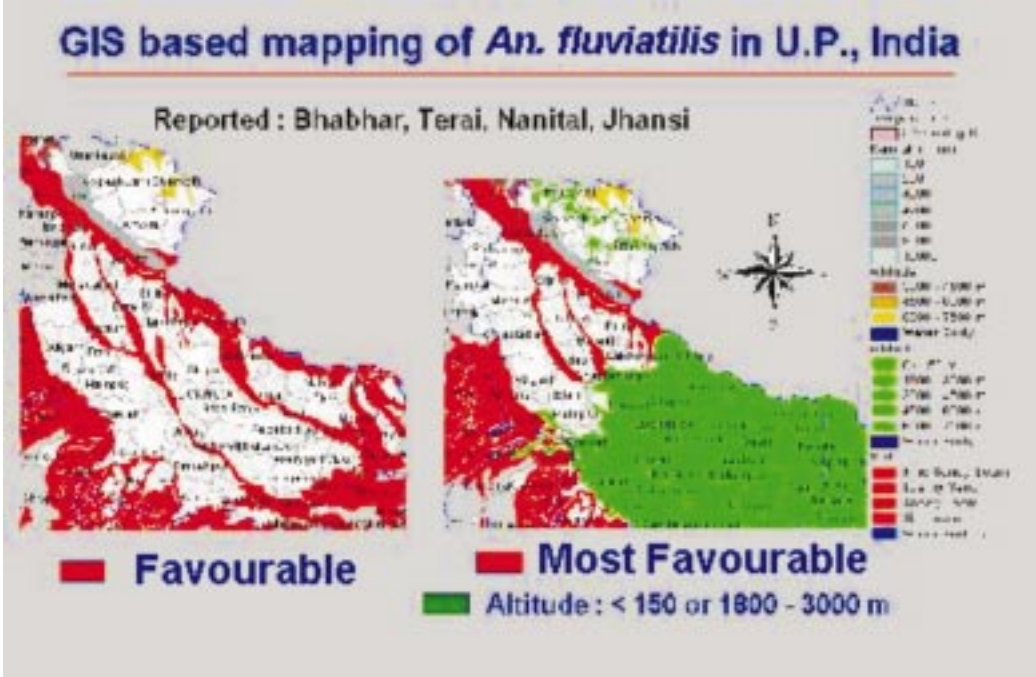


Fig. 4: GIS-based distribution of *An. fluviatilis* using ecological parameters such as soil, altitude, rainfall and temperature

Besides mapping of vector distribution, database consisting of ecological parameters suitable for breeding, survival and longevity for non-vector species has been generated. A software has been developed to identify favourable conditions for individual species. The condition set designed for continuous variables consists of favourable conditions ranging from minimum to maximum whereas for discrete variables individual values were pooled. Thematic maps prepared for vector distribution with species-specific conditions were overlaid and integrated, the resultant maps showed favourable areas of respective species distribution. Reported areas have been overlaid to validate the GIS predicted results. The results are reconciling well with the reported distribution.

The work on 25 species in subgenus *Cellia* namely, *An. kochi, An. balabacensis, An. elegans, An. karwari, An. tessellatus, An. splendidus, An. pulcherrimus, An. jamesii, An. pseudojamesi, An. annularis, An. pallidus, An. philippinensis, An. nivipes, An. jeyporiensis, An. sergentii, An. moghulensis, An. subpictus, An. sundaicus, An. vagus, An. varuna, An. aconitus, An. majidi, An. maculatus, An. willmorei* and *An. theobaldi* has been completed. The work on other four species in subgenus *Cellia* and 23 species in subgenus *Anopheles* is in progress.

Spiracular Indices of *An. stephensi* in an Arid Zone (Rajasthan)

A total of 2944 female specimens of *An. stephensi* were collected indoors during the surveys from 20 villages in three seasons. Out of these 1261, 321 and 1362 specimens were collected during the summer, monsoon and post-monsoon seasons respectively. Out of 2944 specimens, 1779 gravid and semi-gravid mosquitoes were kept individually for egg laying in bowls and 1444 specimens yielded eggs. The numbers of ridges on the egg float were counted and batches of 1156 specimens were identified as *An. stephensi* type form and 288 as *mysorensis*. In type form the ridge number ranged between 15 and 24, while in *mysorensis* 11 and 14, which substantiated the earlier findings. The length of spiracle of type form was found longer than that of *mysorensis*. In type form the average length ranged between 0.11 and 0.12 mm while in *mysorensis* it ranged between 0.09 and 0.1 mm. The difference in spiracle length of type form and *mysorensis* was found to be statistically significant in all the seasons ($p < 0.05$). The spiracular index calculated for *An. stephensi* type form varied from 8.09 to 9.09, while it varied from 6.82 to 7.69 for *mysorensis*. It is noteworthy to mention that difference in spiracular indices were also found to be statistically significant ($p < 0.05$) in all the three seasons. It has been observed that the length of thorax remains constant and it is the length of spiracle which varies through seasons and this holds good for both the variants.

Vinogradaskaya, a Russian scientist, observed that mosquito species having one generation in a year such as *Aedes communis* population in Russia do not show any seasonal variation in spiracular index, but species with multiple generations such as *An. messeae* undergo seasonal variation in spiracular length and index. These indices are low in population during summer (dry period) and increase during spring and autumn when humidity is high. Similar observation has been observed in the case of *An. stephensi* type form and its ecological variant *mysorensis*. *An. stephensi* having multiple generations in a year exhibit significant variation in spiracle length and spiracular indices in different generations which emerge during monsoon and summer seasons. The mosquito population collected during summer season showed smaller spiracle length as compared to the monsoon and post-monsoon population which had longer spiracle length.

Insecticide Resistance

Effect of Piperonyl Butoxide (PB) on the Susceptibility to Deltamethrin in a Selected Deltamethrin Resistant Strain of *An. culicifacies*

Resistance to deltamethrin (a synthetic pyrethroid) in *An. culicifacies* may occur either due to the involvement of mono-oxygenases or esterases or due to a knock-down resistance (kdr) like mechanism, caused by a mutation in the sodium channel gene or may be due to more than one mechanism. The resistance due to mono-oxygenases can be detected by biochemical assays. Alternatively mono-oxygenases-based mechanism can also be detected by pre-exposing mosquitoes to a synergist PB followed by exposure to deltamethrin. To study the effect of PB on selected deltamethrin resistant strain of *An. culicifacies*, PB was treated on Whatman filter papers (12 x 15 cm) in different concentrations — 2.5, 5, 10 and 20% and bioassays were performed by exposing one day old glucose fed *An. culicifacies* to different concentrations of PB alone for one hour. No effect of PB alone up to a concentration of 20%, was observed against *An. culicifacies*. This conc. was further used for bioassays with deltamethrin. Two sets of mosquitoes one without pre-exposure to PB and the other with one hour pre-exposure to PB were exposed to deltamethrin 0.05% for different exposure periods and LT_{50} values were calculated for the two series of bioassays. Results clearly revealed enhanced susceptibility of selected deltamethrin resistant strain to deltamethrin in the presence of PB, indicating the involvement of mono-oxygenases in deltamethrin resistance in this strain of *An. culicifacies* (Table 1; Figs. 5 and 6).

Table 1. Susceptibility of *An. culicifacies* Rameshwaram (Rmr) strain and after selection with deltamethrin and in the presence of PB

Strain	Exposure to		LT_{50} (min)	LT_{90} (min)	χ^2(df)
	Deltamethrin 0.05%	PB 20%			
An. culicifacies Rmr parental	+	–	2.88	8.32	1.20 (4)
An. culicifacies Rmr parental	+	+	1.05	7.99	1.47 (4)
An. culicifacies Rmr (F-14 deltamethrin resistant)	+	–	19.79	135.8	1.42(2)
An. culicifacies (F-14 deltamethrin resistant)	+	+	3.11	26.05	5.01(2)

df—Degree of freedom.

Fig.5: Effect of Piperonyl butoxide on susceptibility to deltamethrin in selected deltamethrin resistant strain of *An. culicifacies*

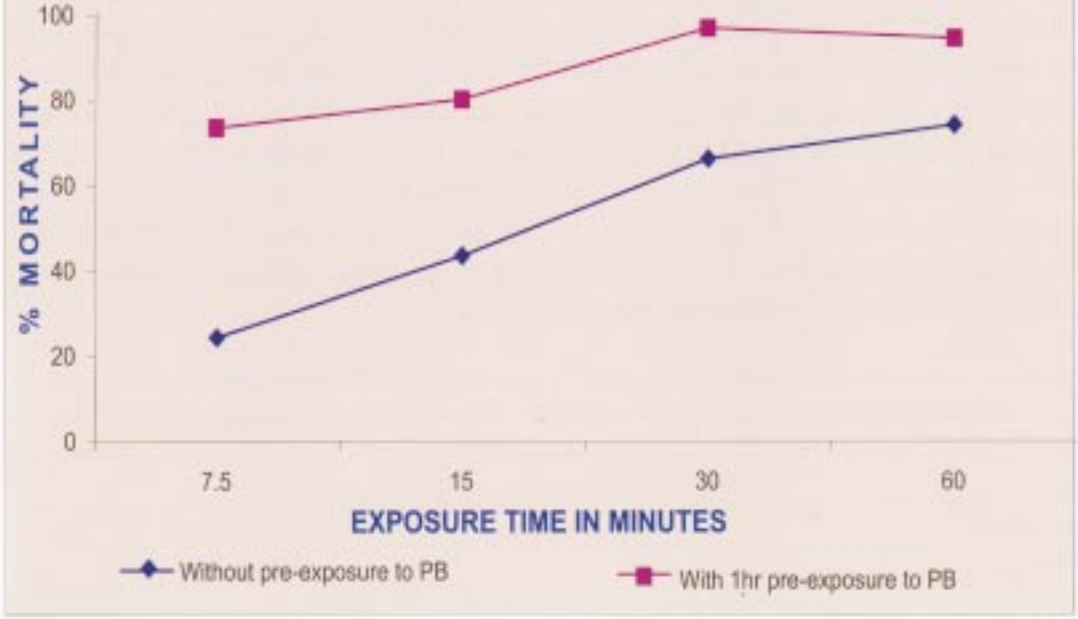
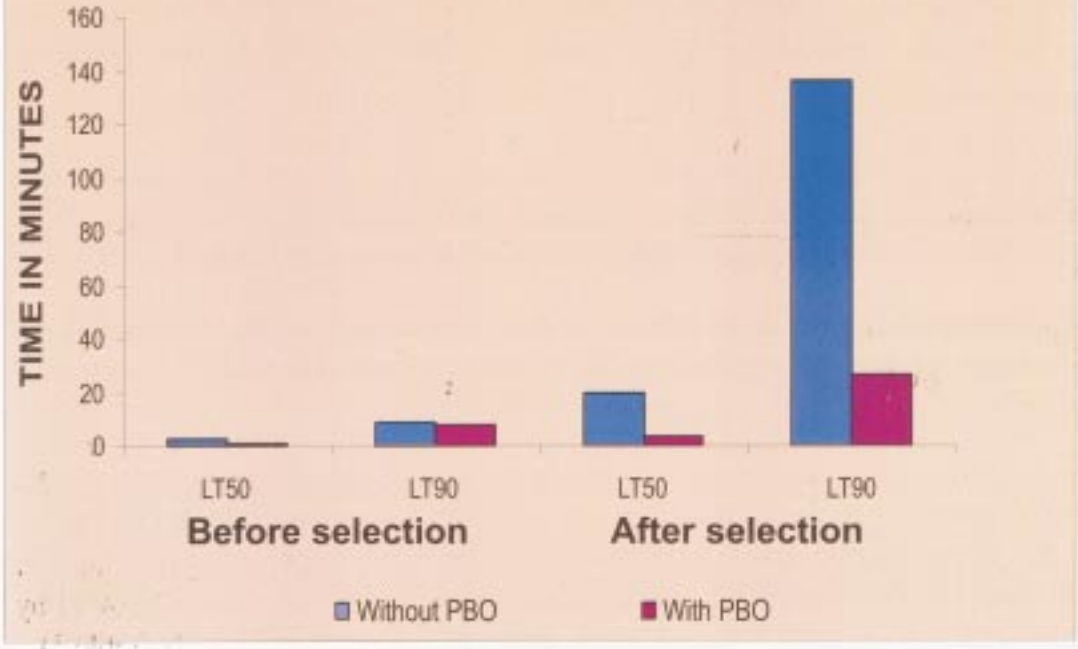


Fig.6 : Susceptibility of *An. culicifacies* Rameshwaram (Rmr) strain to deltamethrin (0.05%) before and after selection with deltamethrin and in the presence of PBO



Susceptibility of Selected Deltamethrin Resistant Strain of *An. culicifacies* to Four other Synthetic Pyrethroids

Susceptibility of selected deltamethrin resistant strain from Rameshwaram was determined against four other synthetic pyrethroids—lambdacyhalothrin, permethrin, cyfluthrin and bifenthrin using insecticide treated papers of discriminatory conc. in WHO test kit. Results of these tests revealed much higher LT_{50} and LT_{90} against all the synthetic pyrethroids as compared to the deltamethrin susceptible strain of *An. culicifacies* (Table 2) and these values were comparable with the LT_{50} obtained for deltamethrin, thus indicating the cross-resistance to other synthetic pyrethroids namely lambdacyhalothrin, cyfluthrin, permethrin and bifenthrin in the deltamethrin selected strain of *An. culicifacies*. However, the resistance ratio in case of permethrin and bifenthrin was much less as compared to the other three synthetic pyrethroids—deltamethrin, lambdacyhalothrin and cyfluthrin (Table 2).

Table 2. Comparison of toxicity (LT_{50} and LT_{90} in min) of different synthetic pyrethroids against deltamethrin susceptible and selected deltamethrin resistant strains of *An. culicifacies*

Insecticide tested	Toxicity				RR at LT_{50}	RR at LT_{90}
	An. culicifacies (Jabalpur) deltamethrin susceptible strain		*An. culicifacies* (Rameshwaram) deltamethrin resistant strain			
	LT_{50}	LT_{90}	LT_{50}	LT_{90}		
Deltamethrin (0.05%)	< 0.5	< 0.5	38.94	97.21	> 77.88	>194.42
Lambdacyhalothrin (0.05%)	< 0.5	0.683	42.83	109.91	> 85.66	160.92
Permethrin (0.75%)	0.76	1.825	38.24	86.25	50.31	47.26
Cyfluthrin (0.15%)	< 0.5	< 0.5	39.94	127.20	>79.88	>254.4
Bifenthrin (0.1%)	1.79	6.75	83.41	221.58	46.59	32.82

Resistance ratio (RR) = LT value of selected strain/LT value of susceptible strain.

Laboratory Evaluation of the Efficacy of *Bacillus thuringiensis* H-14 Formulation Developed by Wockhardt vis-à-vis VectoBac 12 AS and Bacticide

Efficacy of Wockhardt (50%) *Bti* formulation was tested in the laboratory against IV instar larvae of *An. stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* and the results were compared with other two formulations of *Bti*—Bacticide and VectoBac 12 AS, already being used in multicentric field trials by NAMP. The formulation was most effective against *Cx. quinquefasciatus* (LC_{50} = 0.035 mg/l) followed by *Ae. aegypti* (LC_{50}= 0.0628 mg/l) and *An. stephensi* (LC_{50} = 0.221 mg/l) (Table 3).

Table 3. Comparative efficacy of Wockhardt *Bti* formulation vis-à-vis VectoBac 12 AS and Bacticide against mosquito larvae

Mosquito species	*Bti* formulation	LC_{50} (95% confidence limits)	LC_{90}	c^2 (df)
An. stephensi	Wockhardt WP	0.2216 (0.201–0.244)	0.472	14.42 (4)
	Bacticide WP	0.158 (0.139–0.179)	0.5326	10.25 (4)
	VectoBac 12 AS	0.135 (0.12–0.152)	0.419	7.18 (4)
Cx. quinquefasciatus	Wockhardt WP	0.035 (0.032–0.038)	0.0612	2.66 (2)
	Bacticide WP	0.037 (0.033–0.041)	0.0872	10.23 (4)
	VectoBac 12 AS	0.106 (0.095–0.12)	0.329	25.88 (4)
Ae. aegypti	Wockhardt WP	0.0628 (0.057–0.068)	0.126	10.03 (4)
	Bacticide WP	0.0439 (0.139–0.179)	0.1019	1.61 (4)
	VectoBac 12 AS	0.0281 (0.024–0.032)	0.0881	6.92 (4)

df— Degree of freedom.

The efficacy of Wockhardt formulation and other two *Bti* formulations varied against the three vectors. Wockhardt formulation was less effective than VectoBac and Bacticide against *An. stephensi* and *Ae. aegypti* but against *Cx. quinquefasciatus* Wockhardt formulation was most effective of the three formulations.

Vector Control

Bio-efficacy and Operational Feasibility of Alphacypermethrin (Fendona Synthetic Pyrethroid) Impregnated Mosquito Net/Curtains to Control Rural and Urban Malaria [Contract Research Project with M/s. CYNAMIDE]

This study was undertaken as contract research project in 1999 in Jadhonpur and Siddhipur villages of Dhaulana PHC, Distt. Ghaziabad (U.P.) and successfuly completed in 2001. Untreated nylon nets were distributed to all family members in each house in Siddhipur village, while alphacypermethrin treated nets @ 25 g/m^2 were given to the inhabitants of Jadhonpur village. Mubarakpur village located at about 10 km away from the experimental village in Dasana PHC was taken as control where nets were not distributed. Similarly, alphacypermethrin treated jute and cotton curtains were evaluated in Sadiq Nagar, south Delhi.

Results revealed that introduction of alphacypermethrin treated nets in Jadhonpur village considerably reduced the density of mosquitoes particularly of *An. culicifacies* as compared to that in villages where untreated or no nets were distributed. The average density of *An. culicifacies* reduced from 25 to 3 in human dwellings. However, the densities of *An. culicifacies* and *Cx. quinquefasciatus* considerably increased in cattlesheds due to the use of treated nets in human dwellings. Similar results were obtained in urban areas where alphacypermethrin treated jute and cotton curtains were used by the inhabitants.

Results also revealed that the use of alphacypermethrin treated nets drastically reduced malaria transmission. Slide positivity rate (SPR) was nil and 42.8 in treated nets and control villages respectively just after one year of the introduction of nets. This was also reflected when other epidemiological indicators were compared between the control and experimental localities in urban area. *Pf*/000 were nil and 4.4 in treated and untreated curtain areas respectively.

Impact of Residual Spraying of Reldan 40% EC against DDT and HCH Resistant Malaria Vector—*An. culicifacies* in Malaria Endemic Villages of District Ghaziabad (U.P.) [Contract Research Project with M/s. DENOCIL]

This study was initiated in 1999 as a sponsored project and completed in 2001. Reldan 40% EC formulation was sprayed @ 0.5 and 1 g/m^2 in Tatarpur and Chauna villages respectively under Dhaulana PHC of Distt. Ghaziabad (U.P.). The Piyawali village located at about 12 km away in Dadri PHC of the same district was taken as control. Results revealed that insecticide residual spraying (IRS) with Reldan @ 0.5 g/m^2 drastically reduced the density of *An. culicifacies*. Similarly, the Reldan spraying also had a great impact on the prevalence of malaria. There was no significant difference between single and double dose application. However, 1 g/m^2 may be more appropriate for comprehensive vector control.

Impact of Residual Spraying of Bendiocarb 80% WP (Carbamate) against DDT and HCH Resistant Malaria Vector—*An. culicifacies* in Malaria Endemic Villages of District Ghaziabad (U.P.) [Contract Research Project with M/s. HOECHST]

This study was initiated in 1999 with an objective to evaluate the impact of residual spraying of bendiocarb 80% WP @ 0.2 and 0.4 g/m^2 in Nahal and Dehra villages in Dasana and Dhaulana PHCs respectively of Ghaziabad district (U.P.). The Dhulana village located in the same PHC was taken as control. Spraying for three consecutive years produced the desired impact. The density of *An. culicifacies* was dramatically reduced to negligible numbers in both Nahal and Dehra villages even with spray @ 0.2 g/m^2. However, bendiocarb spraying @ 0.4 g/m^2 provided consistent results against *Cx. quinquefasciatus*. Similarly, the malaria incidence particularly the *Pf*

incidence was drastically reduced suggesting thereby interruption of transmission in bendiocarb sprayed villages. The study will be completed in 2002.

Laboratory and Field Evaluation of Hilmilin against Mosquitoes [Contract Research Project with M/s. HIL]

This study was initiated in 1999 and successfully completed in 2001. The results revealed that hilmilin formulations were quite effective in the inhibition of adult emergence at very low dosages. Results of field trials against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* revealed that they are almost equally susceptible to both 25% WP and 22 SL formulations of hilmilin @ 0.004 ppm in variety of habitats with marginal fluctuations. Complete inhibition of adult emergence was observed for 4–7 weeks after application, while persistence of the compound was recorded up to 8–10 weeks. Nevertheless, impact of insect growth regulator (IGR) and other larvicides should be evaluated under a common protocol on adult density and transmission of disease to determine the relative cost-effectiveness and safety to non-target organisms.

Evaluation of the Impact of DDT and Malathion Indoor Residual Spraying being Used in Malaria and Kala-azar Control Programmes on the Disease Prevalence — A Multicentric Study

A nine-month multicentric field study was started in June/July as directed by the DDT Mandate Committee (GOI). The major objective for the study is to evaluate the efficacy of DDT and malathion indoor residual spraying in malaria control. The study areas include four districts for evaluation of DDT spray, namely Bareily (U.P.), Mandya (Karnataka), Chhindwara (M.P.) and Kamrup (Assam) and two districts, Kheda (Gujarat) and Hardwar (Uttaranchal) for malathion spray evaluation. Studies were carried out by the MRC field station staff.

A common protocol was drawn for the study. Target population size in each district was ~ 7000. Insecticide spray operations were carried out under the supervision of the investigating scientists and staff of field stations conducting the studies in the respective study areas. Entomological and parasitological evaluations were done during pre- and post-spray periods on various aspects—vector abundance, sibling species composition, susceptibility to insecticides and disease prevalence. The work is in progress and will be completed in March 2002.

Evaluation of Insect Growth Regulator (IGR) – Triflumuron (OMS-2015) against Larvae of Mosquito Vectors [Contract Research Project with M/s. BAYER (India) Ltd.]

Development of IGRs has been receiving much attention for selective vector control. IGR–"Triflumuron" is a chitin synthesis inhibitor and prevents moulting in aquatic

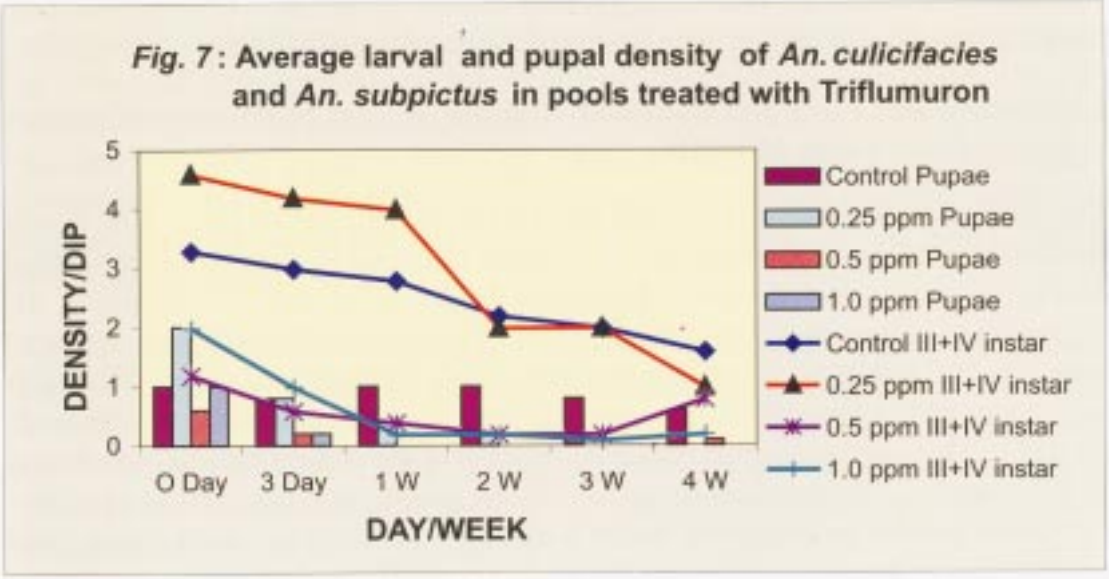


Fig. 7: Average larval and pupal density of *An. culicifacies* and *An. subpictus* in pools treated with Triflumuron

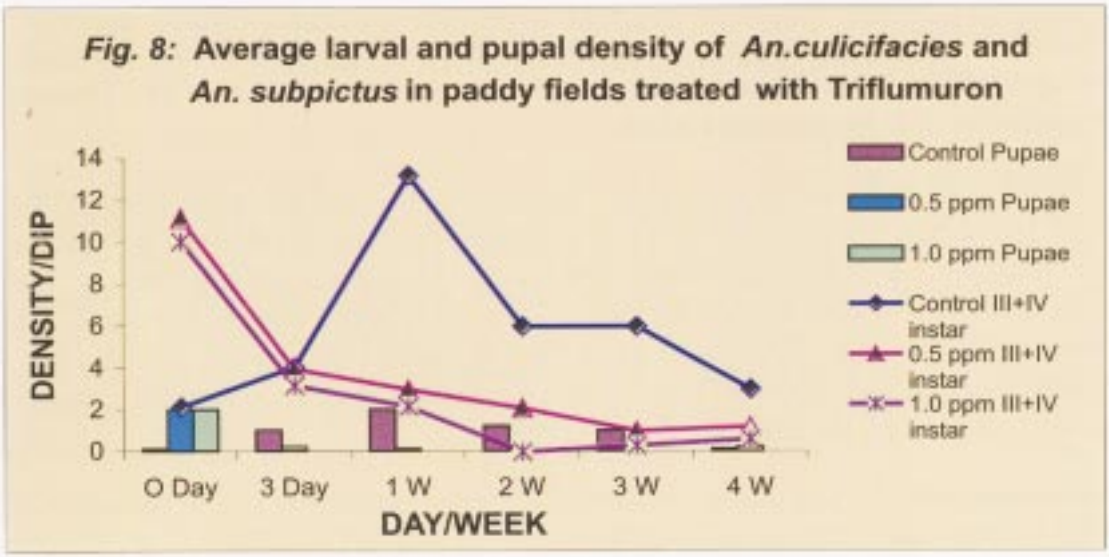
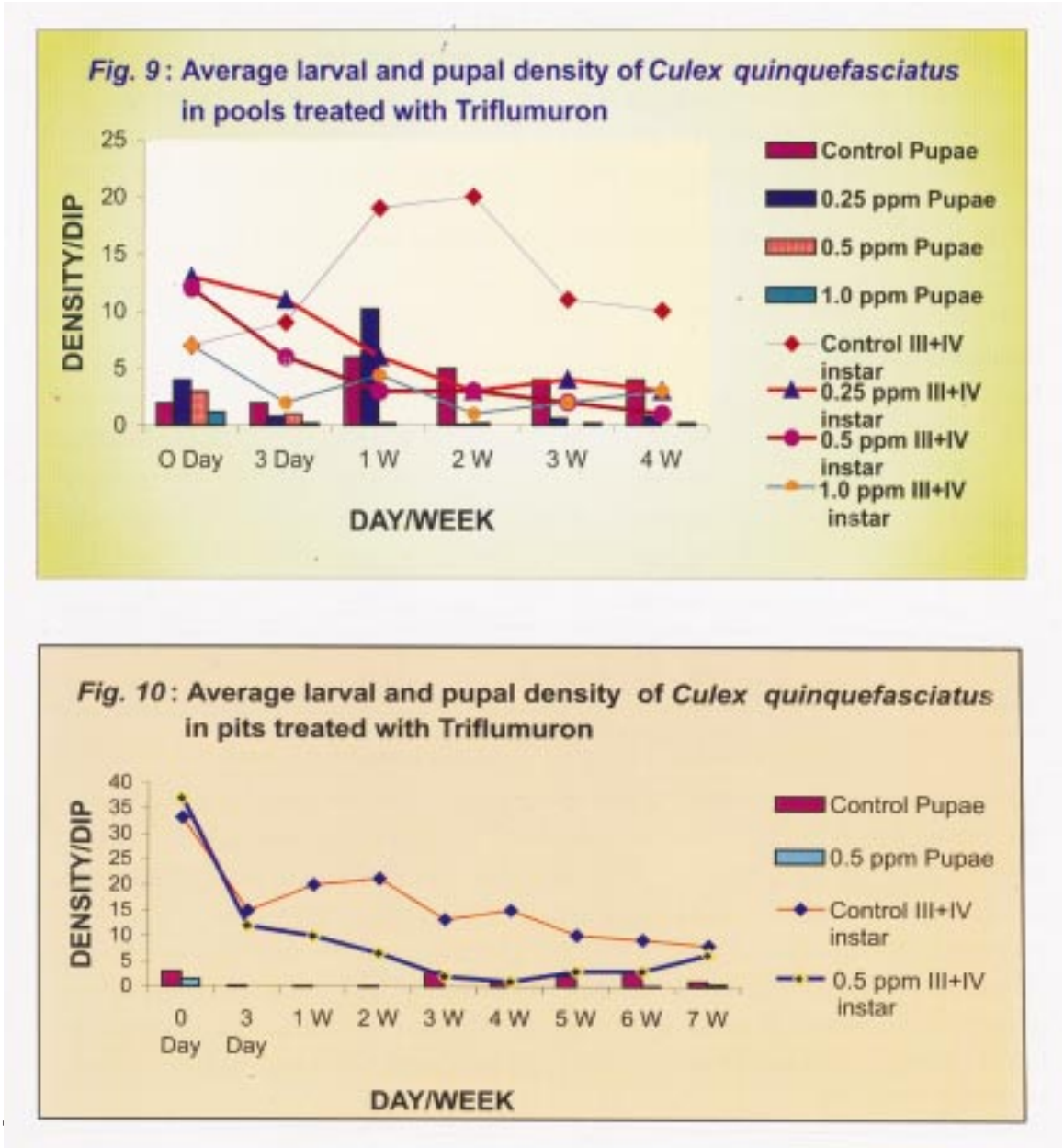


Fig. 8: Average larval and pupal density of *An.culicifacies* and *An. subpictus* in paddy fields treated with Triflumuron

stages of mosquitoes. Starycide SC (suspension concentrate), a Triflumuron formulation was tested in laboratory against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* larvae. Field trials were also carried out in and around Delhi against anopheline and culicine mosquitoes in different breeding habitats. Per cent inhibition of adult emergence was calculated in laboratory against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* and LC_{50} (mg/l) was 0.0001, 0.0003 and 0.0002, respectively. It was found most effective against *An. stephensi* followed by *Cx. quinquefasciatus* and *Ae. aegypti*.

In small scale field trials, this IGR compound was sprayed @ 0.25, 0.5 and 1 ppm in pools and paddy fields against anophelines (mainly *An. culicifacies* and *An. subpictus*)

and density of larvae per dip and that of pupae was monitored. Results indicated the reduction in larval density with all three concentrations and pupal production was completely inhibited after one week up to three weeks when it was used @ 1 ppm concentration (Figs. 7 and 8). In field trials against culicine mosquitoes same doses were used as against anophelines. Trials were conducted in pits, pools and paddy fields against *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*. Results showed decline in larval density and pupal production was nil at 1 ppm concentration for four weeks when used in paddy field and pits. (Figs. 9 and 10).



inhibition potential of this IGR compound, however, conclusion can be drawn only after large-scale field trials.

Studies on Larvicidal Properties of *Solanum nigrum* (Linn.) Seed Extracts

Initial studies with aqueous leaf extract of *Solanum nigrum* (Linn.) were encouraging with complete mortality with 0.2% concentration. Therefore, aqueous and hexane extracts (cold) of dried seeds were used in bioassays against III instar larvae of *An. culicifacies* species A. Results of exposure are given in Table 4.

Table 4. Results of the bioassays with aqueous extract and hexane extracts of dry seeds of *S. nigrum* (Linn.) against *An. culicifacies* species A larvae

Concentration	% mortality (n)		
	24 hours	48 hours	72 hours
Aqueous extract (%)			
0.048	100 (25)	–	–
0.024	100 (25)	–	–
0.012	32 (25)	–	–
0.006	8 (25)	–	–
Hexane extract (ppm)			
100	80 (50)	100 (50)	–
50	48 (50)	60 (50)	76 (50)
25	28 (50)	36 (50)	40 (50)
12.50	12 (50)	20 (50)	26 (50)
6.25	8 (50)	18 (50)	20 (50)
3.125	6 (75)	16 (75)	20 (75)
1.562	0 (75)	12 (75)	16 (75)
0.8	0 (75)	12 (75)	44 (75)

Figures in parentheses indicate total number of larvae treated.

Treatment with aqueous extract of the dried seeds at 0.024% concentration resulted in complete larval mortality and the calculated median lethal concentration (LC_{50}) was 0.0125% and LC_{90} was 0.0208%. With hexane extract complete mortality was at 100 ppm concentration and the calculated median lethal dose (LD_{50}) was 25.50 ppm and LD_{90} was 257.03 ppm. Two more plants (coded SP1 and SP2) were tested for possible larvicidal activity. Crude aqueous extract of leaves was tested in different concentrations against III instar of *An. culicifacies* species A and *Cx. quinquefasciatus* larvae . Both have shown promising larvicidal effect and SP1 has also shown growth inhibiting property.

PARASITE BIOLOGY

Parasite Bank

The parasite bank is involved in the collection and characterization of more field isolates of malaria parasite species from different areas. From Bissamcuttack, Orissa, a total of 22 *Plasmodium falciparum*, five mix (*P. falciparum* and *P. vivax*), four *P. vivax* and two *P. malariae* isolates were collected. These samples were collected from Christian Hospital, Bissamcuttack and tribal villages—Tado and Kakaromaska. Three *P. falciparum* and 17 *P. vivax* isolates collected from Delhi were also cryopreserved in liquid nitrogen (Table 5). Chloroquine sensitivity status of 19 of these isolates was carried out using WHO tests kits. Out of these, three samples collected from Delhi were found to be sensitive to chloroquine and all the 16 samples from Orissa were resistant to chloroquine. Among the 16 samples tested from Orissa, four were resistant up to 64 pmol and three were resistant up to 32 pmol (Table 6).

Table 5. Human malaria parasites collected during 2001

Place of collection	*P. falciparum*	*P. vivax*	*P. malariae*	Mix (*Pf*+*Pv*)	Total
Delhi	3	17	–	–	20
Christian Hospital, Bissamcuttack (Orissa)	9	3	1	1	14
Village–Tado, Bissamcuttack (Orissa)	9	1	–	3	13
Village–Kakaromaska, Bissamcuttack (Orissa)	4	–	1	1	6
Total	25	21	2	5	53

Table 6. Chloroquine sensitivity status of *P. falciparum* isolates

Place of collection	No. of isolates tested	Sensitivity status				
		Sensitive (1– 4 pmol)	Resistant (pmol)			
			8	16	32	64
Delhi	3	3	–	–	–	–
Christian Hospital, Bissamcuttack (Orissa)	6	–	3	–	2	1
Village–Tado, Bissamcuttack (Orissa)	8	–	–	5	1	2
Village–Kakaromaska, Bissamcuttack (Orissa)	2	–	–	1	–	1
Total	19	3	3	6	3	4

Twenty-five *P. falciparum* samples were added this year and at present the total number of *P. falciparum* isolates preserved at the parasite bank is 580. In addition to characterization of *P. falciparum* isolates for sensitivity to antimalarials, 20 isolates were characterized for size variation in MSP 1 and MSP 2 antigens by nested polymerase chain reaction (PCR) assay. A total of twenty isolates collected from Rajasthan, Uttar Pradesh, Delhi, Orissa, Tamil Nadu were characterized. Maximum number of alleles observed were five in an isolate. Multiplicity of infection in the isolates was about 3.15 by MSP 1 and about 1.95 by MSP 2. A total of nine alleles in MSP 1 ranging between 400 and 650 bp and eight alleles in MSP 2 ranging between 450 and 750 bp were observed. The allelic polymorphism observed in the study samples is shown in Fig. 11. Using these two systems 20 isolates were shown to have 19 different types.

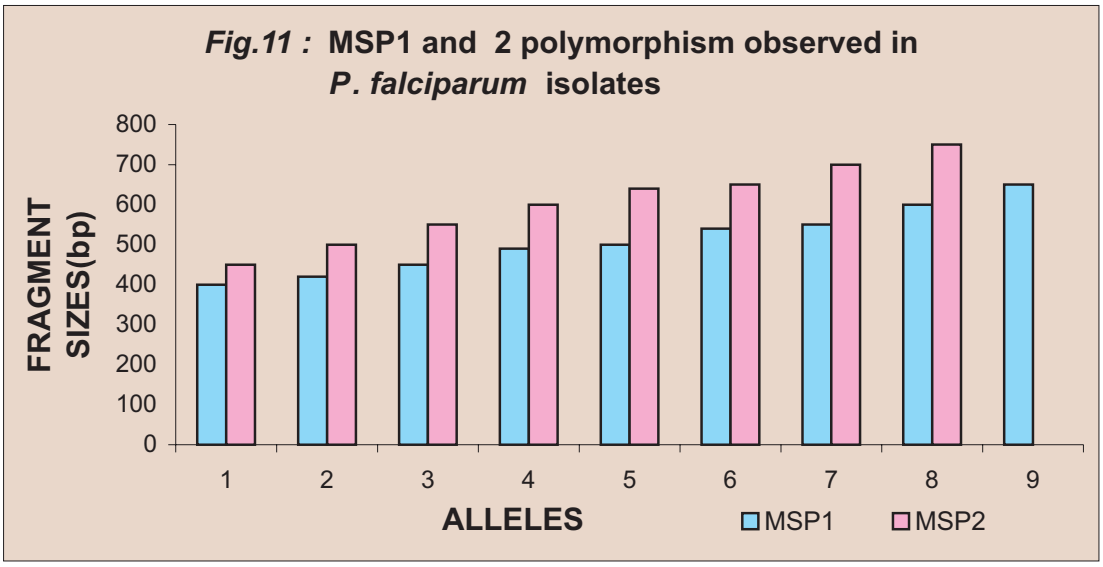


Fig.11 : **MSP1 and 2 polymorphism observed in *P. falciparum* isolates**

Biological materials including non-human and human plasmodia preserved/maintained at the parasite bank were supplied to various research organizations. The non-human parasites, especially *P. berghei* (both chloroquine resistant and sensitive) are being used for the *in vivo* screening of extracts/fractions from medicinal plants. The parasite bank is actively involved in the collection of medicinal plants from various places and in preparing the extracts and fractions for testing their antimalarial activities.

Characterization of Human Malaria Parasites

Plasmodium falciparum

Molecular Analysis of Cytoadherent Phenotype and Invasion Pathways of Indian Isolates of *P. falciparum*

These studies aim to define the cytoadherent phenotypes and the invasion profile of Indian field isolates of *P. falciparum*, collected and cryopreserved at the parasite

bank. The experiments on erythrocyte invasion inhibition using anti-EBA-175 showed encouraging result. Purified anti-EBA-175 RII (F_2) antibody was used to test its ability to inhibit invasion of erythrocytes by the parasites. One laboratory isolate 3D7 and two field isolates, RKL-9 and JDP-8, collected and preserved/maintained at the parasite bank, with known invasion properties were selected for these studies. This antibody raised against EBA-175 showed about 80% inhibition compared to that in controls indicating that this antibody is highly effective in blocking erythrocyte invasion by these parasites. Protein matched serum/pre-immune serum was used as control and the experiments were repeated and the same results were obtained. Four more isolates were characterized for their cytoadherent properties, of which one from Assam and another from Delhi showed high binding to ICAM-1.

Prevalence of Point Mutations in Dihydropteroate Synthetase Gene and *in vitro* Pyrimethamine Sensitivity of *P. falciparum* Isolates Collected from India

The sulphonamide/sulphone, the type-1 antifolate drug is used extensively in the treatment of bacterial diseases as well as in many parasitic infections involving *Cryptosporidium*, *Pneumocystis*, *Toxoplasma* and *Plasmodium*. Members of this group of compounds, such as sulphadoxine and dapsone, inhibit malarial dihydropteroate synthetase (DHPS), a component of the folate biosynthetic pathway. In case of *P. falciparum*, sulphadoxine has been used for prophylaxis. Usually, potentiating mixtures of type-2 (pyrimethamine) along with type-1 antifolates inhibit all growing stages of the parasite. In chloroquine resistant areas the synergistic combination of two antifolates is the alternative treatment for uncomplicated falciparum malaria. *P. falciparum* strains with variable level of sensitivities to sulphadoxine demonstrated sequence variation in DHPS. Sulphadoxine resistant strains showed mutations at 436 Ser to Phe; 437 Ala to Gly; and 613 Ala to Ser or Thr; as well as a single point mutation at 581 Ala to Gly. A further mutation at 540 Lys to Glu was also reported in isolates from Thailand, Bolivia, Kenya and Tanzania.

In the present study the prevalence of point mutations in DHPS gene have been determined at three codon sites, namely 436, 581 and 613 in *P. falciparum* clinical isolates and their *in vitro* sensitivity to sulphadoxine was also tested to draw comparison with molecular assay. Forty finger prick blood samples were collected aseptically from *P. falciparum* patients of all age groups from Assam (n = 15), Ghaziabad (n = 17) and Delhi (n = 8). Primary PCR amplified DHPS gene using two flanking primers yielding a product of 1.15 kb. Amplified DHPS domain from the first round of PCR was used in mutation-specific second round PCR using two flanking and seven mutation-specific primers to detect point mutations at codon sites 436, 581 and 613. Known sulphadoxine sensitive (3D7) and resistant (V1/S) clones were taken as control. *In vitro* assay for sulphadoxine sensitivity was done for all 40 isolates. Parasites were cultured in conditioned media, RPMI-1640 LPLF containing 10%

AB+ serum at a haematocrit of 2.5% and an initial parasitaemia of 0.1–1% in the presence of various concentrations of sulphadoxine (0.4–5000 µM). Parasite growth was monitored microscopically. Rate of schizont maturation as an indicator of parasite growth was calculated for each case and minimum inhibitory concentration (MIC) was determined.

The results of the study confirmed the presence of a few sulphonamide resistant isolates among symptomatic patients and are in agreement with *in vitro* drug sensitivity results. Of the 40 clinical isolates studied, 10% (4/40) presented double mutated forms of S436F and A613T; single mutant type allele A581G was detected in 5% (2/40) isolates. Parasites carrying double or single mutant types showed elevated MIC values above 100 µM (200–515 µM), whereas wild-type parasites sensitive to sulphadoxine were having MIC less than 100 µM (35–100 µM). None of these isolates showed single point-mutation at positions 436 and 613. It is not known to what extent the correlation between molecular techniques and *in vitro* drug sensitivity assay is relevant to the clinical efficacy of sulpha-pyrimethamine; a systematic study on therapeutic efficacy of these compounds at epidemiological level may strengthen the utility of this assay.

P. falciparum Dihydrofolate Reductase Mutation at Thr-108 and Val-16 and Resistance to Antifolate Drug — A Case Study

A patient suffering from *P. falciparum*, who was a traveller to the NE-state from Thailand-Myanmar border, came to Delhi, did not respond to the recommended doses of chloroquine and also to sulpha-pyrimethamine (SP). Although parasitaemia fell rapidly after treatment with SP regimen, the infection had not resolved six days later because of the poor response to treatment selected resistant sub-population. The blood samples collected from this patient prior to treatment with SP regimen and also on Day 2 and Day 6 were tested *in vitro* for antimalarial drug sensitivity and for dihydrofolate reductase gene mutation. *In vitro* drug sensitivity assays demonstrated that the blood samples collected on different days had higher MIC for CQ, pyrimethamine, sulphadoxine and cycloguanil when compared with reference sensitive and resistant strains. Polymerase chain reaction and restriction digest-based methods revealed that the digestion with restriction enzymes Alu I and Bsr I produced a single fragment of 708 bp, whereas with Scr FI produced two fragments of 386 and 322 bp. The Nla III digestion produced two fragments of 568 and 140 bp. The banding pattern with Scr FI and Nla III resembled the mutant FCR3 clone. Overall results on drug sensitivity and RFLP indicated that at the known drug resistant loci, the isolate had a genotype of DHFR Val-16 and Thr-108, previously found associated with cycloguanil resistance. As per the published reports, this type of paired mutations in natural isolates are rare. It is of considerable interest to carry out studies on alleles of this gene in relation to resistance at epidemiological level.

Antimalarial Activity of Amineperoxides *in vitro* in Chloroquine Sensitive and Resistant *P. falciparum* Isolates

Naturally occurring peroxides, such as artemisinin, dihydroascaridole show potent antimalarial activity by oxidative free radical damage to parasite membrane system. The endoperoxide moiety in artemisinin and its analogues is essential for antiparasitic activity which is mediated by activated oxygen or carbon free radicals. The amines containing t-butoxyperoxides show antimalarial activity *in vitro* against *P. falciparum*, but are inactive *in vivo*. A panel of 12 reactive peroxyamines have been synthesized and their chemical structures were determined by a group of researchers under the supervision of Prof. S.V. Bhat of IIT, Bombay. Biological evaluation of these compounds was done by assessing their antimalarial activity *in vitro* in two well adapted *P. falciparum* strains, one chloroquine sensitive and the other resistant. Assay was done at 10% haematocrit containing 1% ring stage parasites in a 96 well flat-bottom tissue culture plate. Compounds were dosed in wells in duplicate at concentrations of 25, 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 µmol per well. Artemisinin was taken as reference drug and also dosed with similar concentrations. To determine the activity of various compounds, assay was done for 24 and 72 hours. The growth of the parasites was monitored microscopically. Percentage schizont maturation and total growth inhibition were calculated to determine the inhibitory concentrations. Nine out of 12 compounds showed antimalarial activity *in vitro*, one compound was found to exhibit maximum antiparasitic activity.

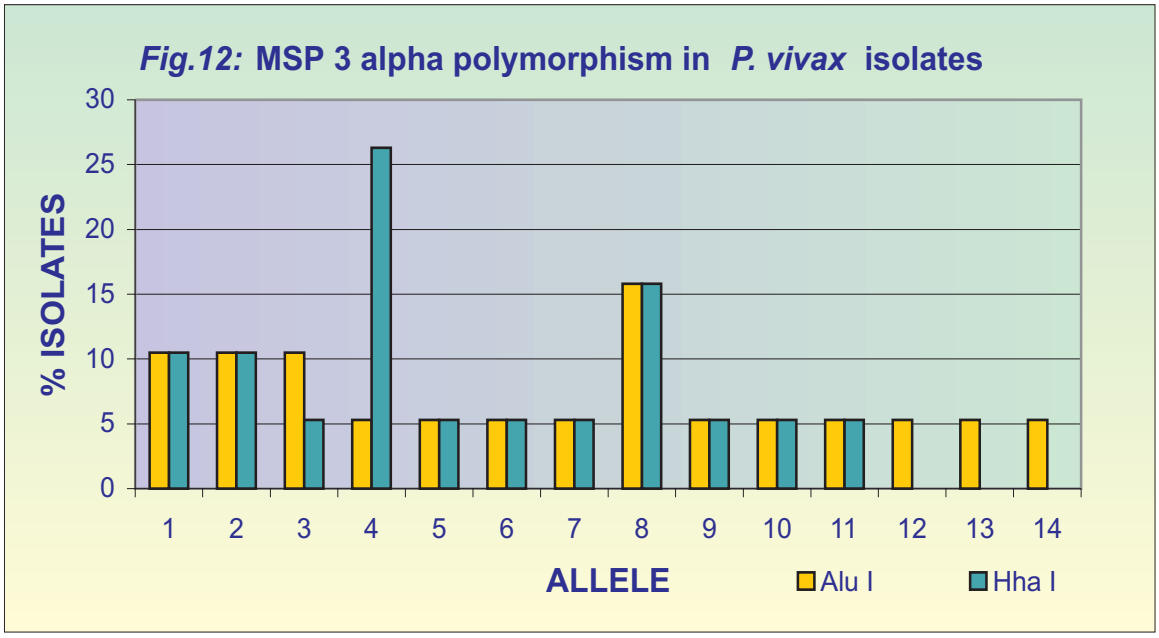
Studies on Genetic Diversity of T-Helper Cell Epitopic Regions of Circumsporozoite Protein of *P. falciparum* Isolates from India

In a previous study on genetic polymorphism of T-helper cell epitopic regions (Th-2R and Th-3R) of CSP of 41 *P. falciparum* isolates from different geographical regions of India, it was found that the T-helper cell epitopes could be categorized into four groups. Both Th-2R and Th-3R variants could be categorized into four groups. Some of the groups categorized based on the sequence variation in Th-2R and Th-3R showed homology with the sequence reported previously. The variations were regionally unbiased as different islolates collected from different regions showed identical sequence variations and belonged to the same group. However, few isolates showed random polymorphism and could not be categorized into groups. In continuation of this study 12 more isolates collected from different geographical regions (Rajasthan, Madhya Pradesh, Delhi and Assam) were studied. Sequence variations in Th-2R and Th-3R showed that 11 isolates exhibited sequence homology with the four groups categorized in the previous study and one isolate showed random variation. Variations in Th-2R and Th-3R in the present study were also found to be regionally unbiased.

Plasmodium vivax

With an aim to select suitable and highly polymorphic markers, isolates collected at malaria clinic, Nanak Enclave, Delhi and samples collected during epidemic investigation in Gautam Budh Nagar, U.P. were analyzed for polymorphism of MSP 3α and DBP (Duffy binding protein).

MSP 3 alpha : A total of 19 isolates were analyzed by PCR-RFLP assay for size and sequence variations. Size variations were observed in the range of 1.2 to 1.8 kb and RFLP assay with Alu I and Hha I has shown changes in the restriction site positions in the alleles. A total of 14 alleles by Alu I digestion and 11 alleles with Hha I digestion were observed. Fig. 12 shows distribution of alleles among the isolates. Among 19 isolates, a total of 16 different genotypes were observed.



Sequence diversity in DBP RII region : With an aim to study sequence diversity in *P. vivax* DBP RII in the Indian field isolates (*P. vivax* duffy binding protein region II has been used in vaccine development), primers have been designed to amplify RII region of DBP from Indian isolates. With these primers, PCR assay has been standardized using genomic DNA and a fragment of about 1 kb has been amplified. Further, amplification, purification and cloning was done for four DNA samples isolated from blood spots collected from *P. vivax* positive patients. Sequencing of the clones is under progress.

Studies on Monoclonal Antibodies against *P. vivax* Erythrocytic Stages

A panel of fifteen hybridomas was raised, eight of them were cultured for harvesting a large volume of supernatant. All of them showed reactivity by IFA test in *P. vivax*

smears with varying degree of fluorescence intensity. By ELISA test, out of 8 lines, 7 were IgG type; one showed positivity for both IgG and IgM. Clones secreting IgG type antibody gave strong signal in IFA and higher optical density in ELISA. Immunoglobulin G isolated from these supernatants were coupled with peroxidase and tested by dot-blot assay on *P. vivax* blots to check the reactivity in clinical specimens. Antibodies showed detection limit in patient's blood having parasitaemia above 0.05%.

In continuation of earlier work, one IgG1 type antibody producing clone was taken for production of large quantities of antibody. On western blot, this monoclonal antibody reacted with a protein above 30 kDa of *P. vivax* crude erythrocytic stage antigen. N-terminal sequencing of the protein was done at IMTECH, Chandigarh. The sequences produced with significant alignments were homologous to one *P. falciparum* cDNA library, *P. berghei* 34 kDa phosphoprotein mRNA and to mouse blastocyst cDNA clone. Since the protein preparation was not completely pure, overlapping of amino acids in the sequences has been observed. This antibody was coupled with horse-radish peroxidase enzyme. The reactivity of the antibody-enzyme conjugate was tested in 20 clinical isolates by dot-blot ELISA. The colour signal was strong with *P. vivax* positive blots.

Biochemical Characterization and Expression of *P. vivax* Aspartic Proteases

The key role of an aspartic protease in initiating haemoglobin digestion in food vacuoles has been defined and two aspartic haemoglobinase I and II (plasmepsins) have been purified in *P. falciparum*. No work has till date been reported on the isolation and characterization and role of aspartic proteases in *P. vivax*. Inhibition of haemoglobin catabolism or other essential functions catalyzed by aspartic proteases in the parasite offers attractive targets for therapeutic intervention studies.

In continuation of our earlier studies, samples from Chennai were processed for the aspartic protease activities in *P. vivax* parasites. Gelatin gel PAGE and ELISA have been used to characterize and assay the protease activities. Two aspartic protease activities have been identified in *P. vivax* having a molecular weight of 37 kDa and 40 kDa which were found to be similar to the activities of plasmepsins in *P. falciparum*. The aspartic protease activities in parasite samples were identified using specific inhibitor namely pepstatin A which shows IC_{50} at 3 µM. The pH dependence of the aspartic protease activity has shown a pH maxima of 4. Kinetic analysis of the inhibition of the enzyme aspartic proteases by Line weaver double reciprocal plot has shown that pepstatin A is a competitive inhibitor of the enzyme with respect to the substrate concentration.

Experiments were initiated to characterize the aspartic protease gene in *P. vivax* using the known primers of *P. falciparum*. However, it was not possible to amplify the *P.*

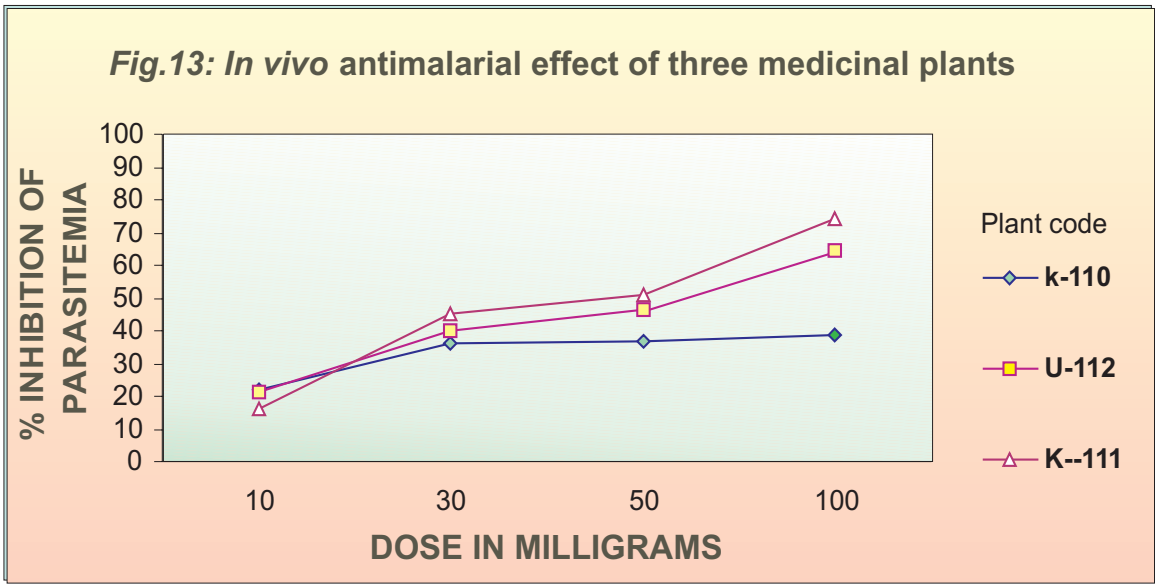
vivax gene using known primers of *P. falciparum*. It is now planned to identify and characterize aspartic protease gene in *P. vivax* using degenerate primers synthesized from the N-terminal amino acid sequences of the plasmepsins from *P. vivax* clone and express it in *E. coli*, purify and refold the recombinant protein for further studies on its role in parasite functions.

Primary Screening of Herbal Products for their Antimalarial Activity

Our earlier studies on the *in vivo* effect of the crude extracts of *Azadirachta indica*, *Phyllanthus niruri*, *Ocimum sanctum* against *P. berghei* showed encouraging results. Studies on this aspect was continued and the antimalarial effect of ethanol extracts of more medicinal plants was studied.

The medicinal plants or parts of the plants used for the treatment of fever in rural/ tribal areas were collected from different geographical regions of India and tested for their antimalarial properties. Fifty per cent ethanol extracts of nine medicinal plants were tested *in vitro* for their antimalarial activity. Some of these extracts, which were showing encouraging results *in vitro*, have been tested *in vivo* this year. The plants were collected, washed and dried in shade and powdered before the preparation of their extracts. The standard methods were followed for the preparation of 50% ethanol extracts using a soxhlet apparatus. The extracts were concentrated and lyophilized for testing their antimalarial properties. The *in vitro* test was done using both chloroquine sensitive and resistant isolates of *P. falciparum*. The assay used was schizont maturation inhibition assay. The *in vivo* test with three medicinal plants was carried out following Peter's 4-day test in which different concentrations of the plant extracts were given orally to the batches of mice after inoculating *P. berghei* intraperitoneally to mice on Day 0. Three more doses were given to mice and on Day 4 slides were prepared from all the mice to quantitate the percentage parasitaemia.

***Fig.13: In vivo* antimalarial effect of three medicinal plants**



The control animal received only parasites. The percentage inhibition of parasitaemia was calculated against the parasitaemia of the control to see the antimalarial effect of the plant products (Fig. 13). All the plants tested showed varying degree of antiplasmodial effect.

These extracts were further fractioned with chloroform, butanol, hexane and ethyl acetate to get the most effective fractions. These studies are in progress. Besides these, four more new plants collected from Kerala, Uttaranchal and Assam have been tested *in vitro* for their antimalarial activity. The antimalarial effect of these plants was not as good as that of the plants tested in our laboratory earlier.

EPIDEMIOLOGY

Malaria Clinics

At MRC, 2 Nanak Enclave: A total of 1993 patients attended the malaria clinic located at 2, Nanak Enclave during January to December, of which 70 were found positive for malaria. Among these, 58 were positive for *P. vivax*. Age-wise distribution of *P. vivax* cases is as follows: 1–4 yrs – 1; 4–9 yrs – 1; 9–14 yrs – 5; and >14 yrs – 51, while 12 *P. falciparum* cases were in the following age groups, 4–9 yrs – 1; 9–14 yrs – 1; and >14 yrs – 10. Slide positivity rate (SPR) for the year varied between 0.79 and 5.56 in different months with an average SPR of 3.51. Case history of *P. falciparum* cases revealed that majority were imported in nature, majority of these patients were natives of Jharkhand and Bihar who visited their relatives in Delhi for seeking employment. The month-wise distribution of these cases are given in Table 7.

Table 7. Data from Malaria Clinic, MRC, Nanak Enclave, Delhi

Month	BSE	Total (+)ve	*Pv*	*Pf*	SPR	SfR
Jan	72	4	3	1	5.56	1.39
Feb	91	1	0	1	1.1	1.1
Mar	145	2	2	0	1.38	0
Apr	127	1	1	0	0.79	0
May	108	8	8	0	7.41	0
Jun	98	5	5	0	5.1	0
Jul	408	17	11	6	4.17	1.47
Aug	271	9	7	2	3.32	0.74
Sep	196	7	7	0	3.57	0
Oct	221	11	10	1	4.97	0.45
Nov	147	2	2	0	1.36	0
Dec	108	3	2	1	1.85	0
Total	1993	70	58	12	3.51	0.6

From the clinic, blood samples were collected from volunteers for the following:

Parasite Bank: Eighteen *P. vivax* blood isolates were deposited at the parasite bank for further studies.

Host-parasite interaction : Forty blood isolates of *P. vivax* and four blood isolates of *P. falciparum* were used for artificial feeding experiments. Among these the

mosquitoes fed on 28 *P. vivax* blood isolates were found positive for both gut and gland infections, while mosquito infectivity could not be obtained when fed on any of *P. falciparum* isolates.

Genetic diversity: Twenty-three *P. vivax* blood isolates were used for MSP3α and duffy binding protein diversity studies.

At MRC, 22 Sham Nath Marg: A total of 210 patients or referred cases from hospitals attended the malaria clinic for blood examination and treatment of malaria during January to December. Out of 38 patients found positive for malaria, 20 were diagnosed as *P. vivax* and 18 as *P. falciparum* cases.

Clinical Studies on Malaria

A study was undertaken with the objective of studying the clinical profile in malaria and to study the level of drug resistance *in vivo* in view of increasing resistant strains of *P. falciparum* and *P. vivax*. Patients with proven infection with *P. falciparum* and *P. vivax* willing to attend the clinic for 28 days follow-up were included in the study (34 *Pv* + 4 *Pf*). Thirteen per cent of them were children. The parasite density ranged from 160 to 52,800 and 400 to 88,320 among 34 *P. vivax* and 4 *P. falciparum* patients, respectively. History of travel to endemic areas (Bihar and U.P.) was found in 50% of the cases. Fever and headache were the main presenting symptoms. All patients were treated with chloroquine dosage and 28 days follow-up was done to assess the drug response. All the 34 *P. vivax* patients had parasite clearance by two days and did not report with recrudescence. Out of 4 *P. falciparum* patients, 3 had adequate clinical response, and one had late treatment failure.

Tolerability and Efficacy of Artesunate plus Chloroquine or Sulphapyrimethamine Combinations vs Single Agent Chloroquine or Sulphapyrimethamine for the Treatment of Uncomplicated *P. falciparum* Malaria

Combination therapy is based on the synergistic or additive potential of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite. The objective of combining antimalarial drugs is to improve efficacy and delay the development and subsequent selection of drug resistant parasites, thus prolonging the useful therapeutic life of drugs in the combination. The advantages of artemisinin-based combination therapy (ACT) relate to the unique properties and mode of action of the artemisinin component, which include rapid reduction of the parasite biomass, rapid resolution of clinical symptoms, effective action against multidrug-resistant *P. falciparum* and reduction of gametocyte carriage rates (which may reduce transmission of resistant alleles specially in the areas with low or moderate malaria transmission). Therefore, studies have been planned to evaluate the role of combination therapy in malaria in Indian scenario.

A study was conducted on microscopically confirmed patients of uncomplicated *P. falciparum* malaria in Mandla, Madhya Pradesh, where resistance to first/second line drugs has been reported. Subjects fulfilling inclusion criteria were enrolled. Safety and tolerability of combination therapy was first established in 12 patients by recording detailed physical, haematological and biochemical parameters before and after the treatment. Subsequently, the patients were enrolled randomly by open design in different treatment groups (monotherapy or combination therapy). Clinical and parasitological parameters were evaluated during 28 day follow-up period. The number of adult patients enrolled and treatment given up to December 2001 are given in Table 8.

Table 8. Number of adult patients enrolled and treated

Group	No. of patients	Drug	Dose (mg)	Treatment schedule
A1	10	Sulphapyrimethamine	1500+75	Day 0
A2	16	Sulphapyrimethamine +	1500+75 +	Day 0
		Artesunate	100 (twice a day)	Day 0, 1 and 2
B1	10	Chloroquine	600+600+300	Day 0, 1 and 2
B2	10	Chloroquine +	600+600+300+	Day 0, 1 and 2
		Artesunate	100 (twice a day)	Day 0, 1 and 2

None of the patients had early parasitological or clinical failure. No major adverse effects were observed in any of the groups. The follow-up and analysis of results is in progress.

Role of Asymptomatic Carriers in the Transmission of Malaria

Parasitological surveys for the detection of asymptomatic carriers of malaria were conducted in District Sundergarh of Orissa. Surveys were carried out in March, June and September 2001. Asymptomatic carriers constituted 40-50% of the total examined in different study areas. Gametocyte density was more in symptomatic cases as compared to that of in asymptomatic carriers. Blood samples drawn from asymptomatic gametocyte carriers were fed to *An. stephensi* in different batches. Two out of 20 samples from asymptomatic carriers showed development of malaria parasites in the vector. On the other hand out of 11samples from symptomatic cases, three showed development of parasites in the mosquitoes. The results showed that the role of asymptomatic gametocyte carriers has to be established in the transmission of malaria. There is an urgent need to develop a test which is simple and could screen large number of samples within a short time. Hence, a peptide for detection of gametocyte antibodies has been prepared and is in the process of standardization.

Spatio-temporal Dynamics of Malaria in Mewat (Funded under ICMR Task Force Project on GIS and RS)

Ground verification with respect to classification of Landsat satellite image of August 1998 was done in the month of August and it was found that the areas falling in Class Bare 1 were highly water-logged during this year also and the malaria incidence and mosquito nuisance in these areas were very high (Fig. 14).

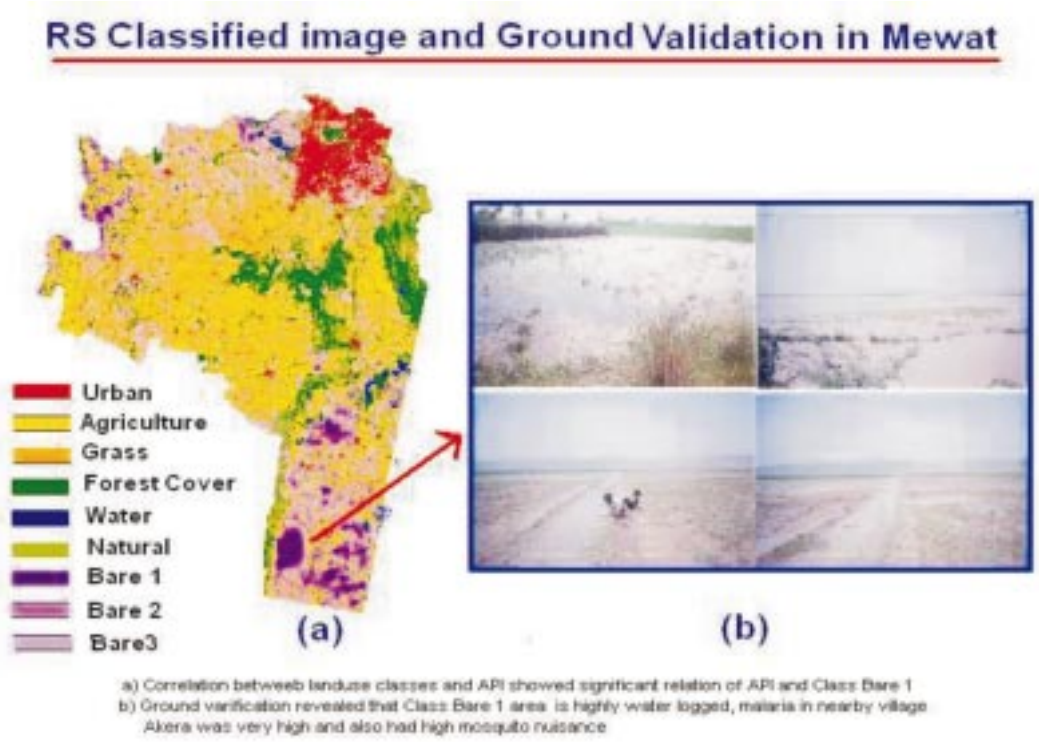


Fig. 14: Ground validation revealed that Class Bare 1 areas are highly water-logged, malaria incidence and mosquito nuisance is very high in nearby villages

A 3D model of Mewat was developed using spot heights and slope map generated by Haryana Remote Sensing Application Centre (HARSAC), Hissar (Fig. 15). Broadly, the area comprised of Nuh, Nagina and Punhana blocks is sandwiched in two ranges of hills. Road network and the village boundaries were draped to identify route for ground verification. The topological features developed by GIS were found to be correct.

Draping of natural drainage network on 3D model and estimation of drainage density could identify the areas potential for water-logging. Drainage density showed a negative correlation with section-wise average API, which shows that the villages where drainage density is high, malaria is low (Fig. 16). Southern and western parts of Mewat area has a slope towards Kotla lake. In addition, the area is drained by Landoha, Ujjina, Nuh and Kotla drains. These drains fill the Ujjina natural depression

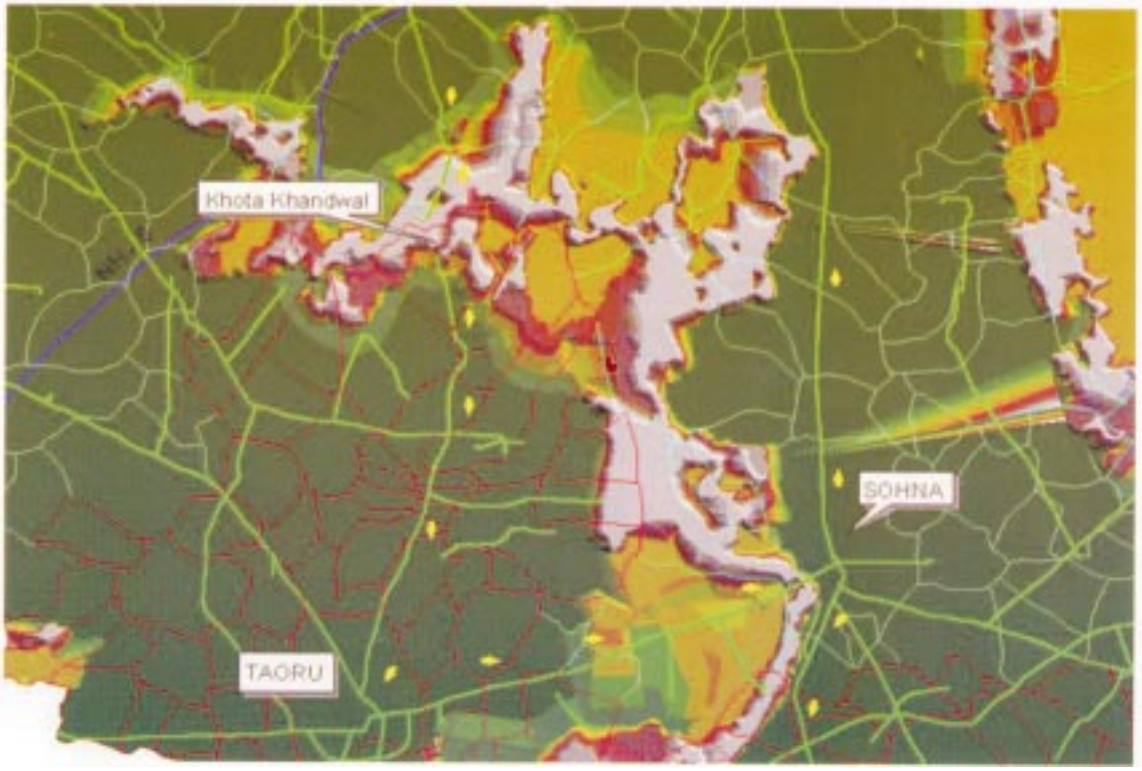


Fig. 15: A 3D model developed using contours and spot heights shows undulations in Mewat region

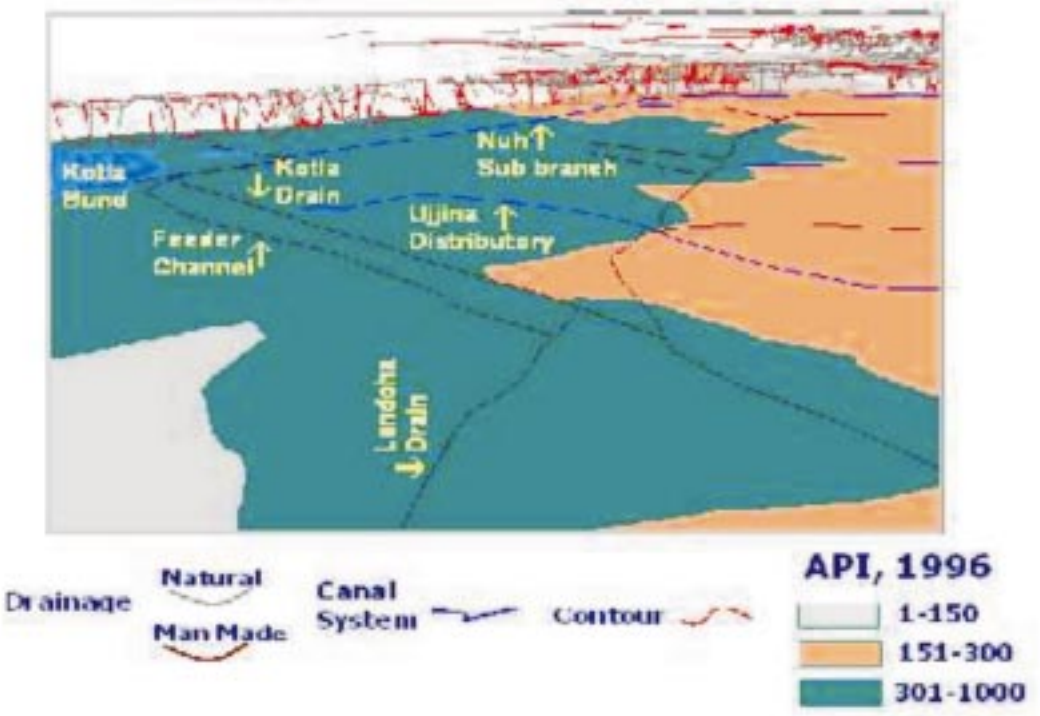


Fig. 16: Drainage and canal system draped on contours of Mewat shows malaria incidence is low in northern area where the drainage density is high

turned lake. Flow direction and water accumulation points were mapped using hydrological modelling features of GIS software. Overlying settlement map of Mewat region showed villages in low-lying areas. Canal network map was also prepared which showed that the part of Mewat region is covered with a network of surface irrigation through network of distributories/ minors originating from Gurgaon canal. Geographical reconnaisance (GR) revealed that extensive seepages originating from distributories etc. find their way to the Kotla lake but before entering, these raise the sub-soil water within 3 m, as a result most of the Mewat region is water-logged.

On the basis of ecological features five malaria paradigms have been identified in Mewat—mining, irrigation command area, flood prone area, catchment area (Kotla and Ujjina lake and surrounding area) and urban area. Villages falling in each paradigm have been identified. Section-wise malaria data for each paradigm have been extracted. Sections with persistent high malaria were identified. The paradigm maps will help in identifying risk factors.

GIS-based Malaria Information Management System for Urban Malaria Scheme in India

GIS based malaria information management system developed for Dindigul town, District Dindigul, Tamil Nadu was updated to cater the ubiquitous needs of Urban Malaria Scheme in India. The system can help in: (i) identifying high receptive areas in time and space domain; (ii) identifying risk factors for high receptivity; and (iii) monitoring and evaluating control measures.

Four proformae for recording information on different epidemiological and entomological parameters (to be used) were developed to update GIS database. This was done in consultation with state and district health officers. The Proforma 1 is for collection of information on key containers, positive breeding sites, larval density, etc. in each street for each habitat. Adult density may be recorded in the Proforma 2, antilarval activities in the Proforma 3. The Proforma 4 is for recording parasitological information. The basic objective was to develop the model to assist planning and implementation of a suitable control measure.

Delineation of Breeding Habitats and Landscape Features Suitable for *An. culicifacies* Abundance in Tumkur district, Karnataka Using Satellite Data (Funded under ICMR Task Force Project on GIS and RS)

In continuation of entomological and landscape ecological data collected and reported earlier in July 2000, entomological data on adult *An. culicifacies* density; prevalence and larval density in breeding habitats in 30 villages (10 each in high, moderate and non-malarious areas) were collected in January 2001 (non-transmission season of

malaria) and in May 2001 (peak season of malaria). Satellite data (IRS LISS III and PAN data) of respective areas in respect of April 2000 (data of July was not available) and December 2000 were analyzed for delineation of breeding habitats, land use features and correlation of land use features with mosquito density. A buffer zone of 1.5 km radius around each village was created and supervised classification of land use features was done. Vegetaion index in entire Tumkur district for April 2000 period was also generated.

It was found that with IRS PAN data, delineation of streams, ponds and tanks was possible but irrigation wells particularly covered under canopy of coconut plantation were not identifiable. Landuse features of three categories of PHCs were compared vis-a-vis breeding potential and man hour density of *An. culicifacies.* The preliminary findings indicate that coconut/arecanut plantation, agricultural land, type and extent of water bodies, less barren area are some of the landscape features supporting *An. culicifacies* abundance in high malarious area.

Meteorological Variables and Malaria: An Analysis for Developing Model of Malaria Transmission (A Project on 'Impact of Climate Change on Human Health— Malaria and Dengue' co-sponsored by Govt. of India and U.K. w.e.f. 1.9.2001 at National Physical Laboratory, New Delhi. MRC is also a participating institute)

In continuation of earlier work in respect of Surat district, Banaskantha (Gujarat) and Bikaner district (Rajasthan) were selected in consultation with NAMP for analysis of meteorological parameters and incidence of malaria from predictive value point of view. Based on optimum conditions required for transmission (Temp. 24ºC and RH 55%) the threshold time of transmission was determined. Month-wise time series analysis of data on temperature, rainfall, *P. vivax* and *P. falciparum* cases for a period of seven years including the period of outbreak of malaria was done. It was found that in Banaskantha district from 1985 to 1987 (drought years), *P. falciparum* cases were only 131 in 1987, while in high rainfall years, .i.e. 1988 –1991, there was an increase in *P. falciparum* cases reaching up to 23201 in 1990 (Figs. 17). The impact of rainfall on malaria incidence was also analysed for Bikaner and Surat districts (Fig. 18). In order to see the impact of rainfall at the threshold of transmission period in a particular year, it was found that high (unusual) rainfall at the beginning of transmission season may be helpful in prediction of malaria in ensuing month.

Testing of Coded Serological Samples for Analysis and Stratification of Three Sites

A total of 760 samples were collected from three different sites of the country during February. The samples were coded and subjected to analysis.

Fig.17: **Impact of meteorological data on incidence of malaria in Banaskantha**

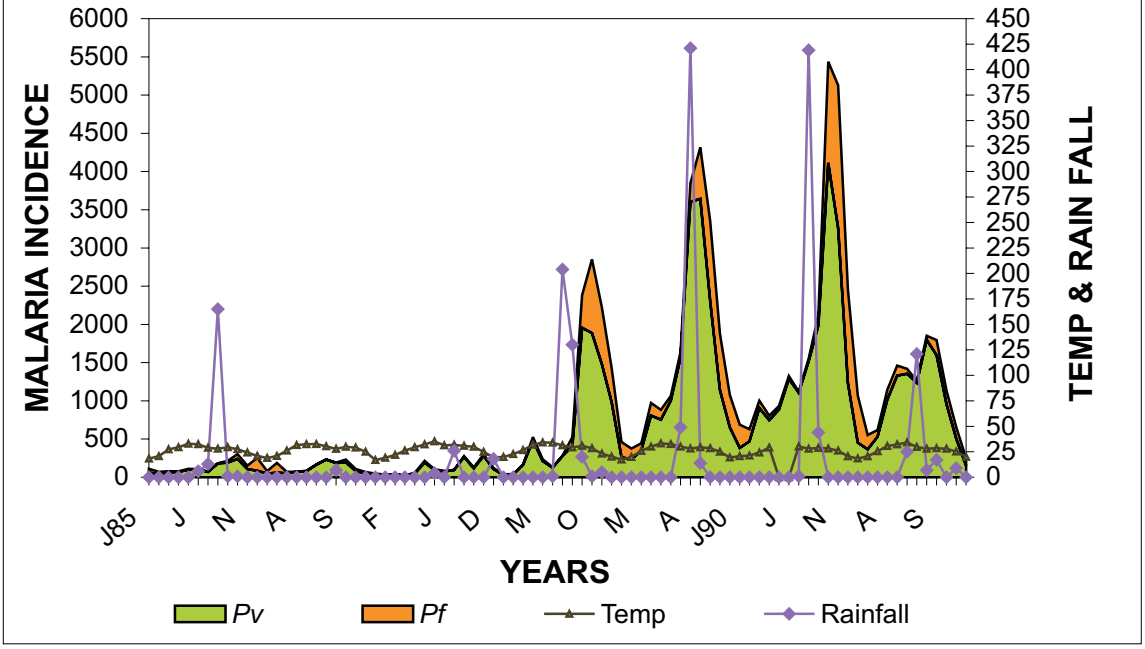
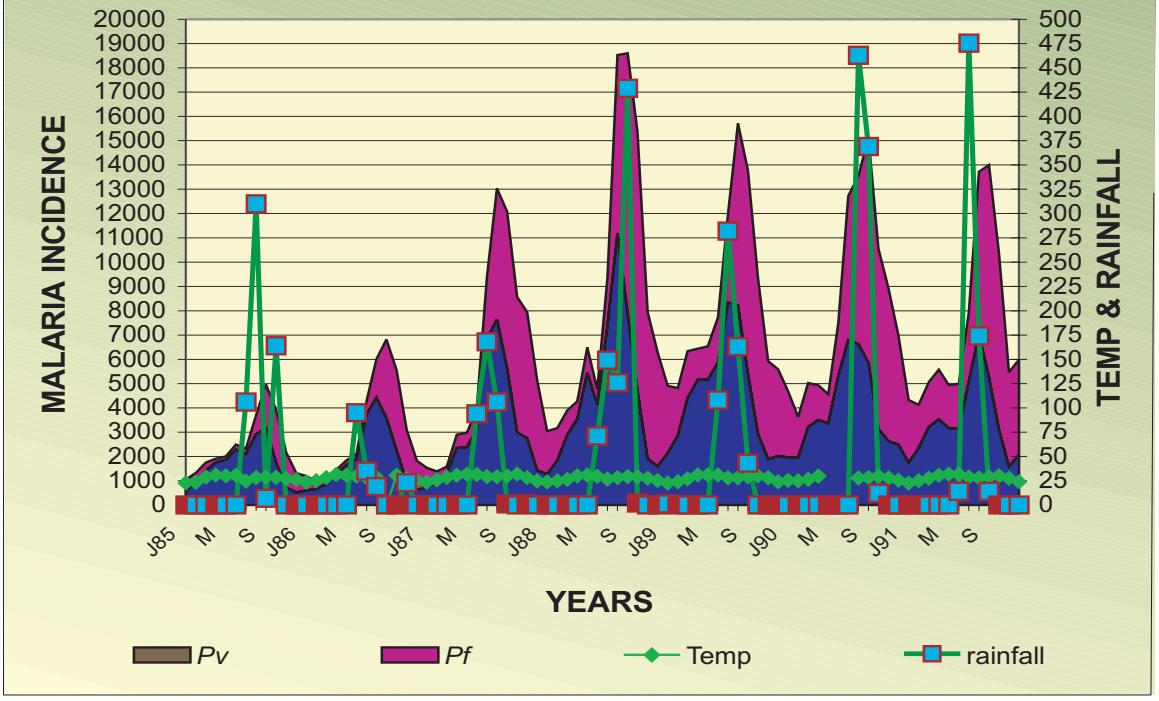


Fig.18 **: Impact of meteorological data on incidence of malaria in Surat**



Site A comprised of three villages in District Shahjahanpur where 306 finger prick blood samples were collected from these villages and no malaria positive case was recorded at the time of sample collection. PHC data from 1997 to 2000 indicated low or no transmission. The samples were divided into three age groups as shown in Table 9. ELISA O.D. obtained from AR1 peptide ELISA were taken into consideration.

Table 9. Analysis of blind samples

Area	Malaria status	Mean O.D.±S.D.		
		1–4 yrs	5–14 yrs	>15 yrs
Site A	Medium	0.47 ± 0.25 (17)	0.45 ± 0.27 (105)	0.37 ± 0.27 (153)
Site B	High	0.85 ± 0.36 (44)	0.86 ± 0.35 (106)	0.83 ± 0.32 (161)
Site C	Low	0.17 ± 0.07 (8)	0.27 ± 0.19 (60)	0.28 ± 0.16 (71)

Figures in parentheses indicate number of samples tested.

Site B comprised Rourkela area which had a history of high malaria transmission as reflected from the PHC data. ELISA O.D. of all the age groups from all study villages indicated high malaria incidence. From six villages of Kuarmunda PHC, 299 samples were collected, including 49 from malaria positive cases. API during the year 2000 in these villages was high and ranged from 23.2–105.4. ELISA O.D. confirmed the parasitological data that malaria transmission is high in this site.

Site C comprised of one village named 'Purum' of Kamasamudram PHC, Kolar district, near Bangalore, and 139 samples were collected. None was found pos-itive for malaria parasite. PHC data for the past three years showed 'zero' API indicating no malaria transmission. ELISA O.D. were less than 0.3 confirming a low transmission.

To confirm serological data with equivalent transmission index (ETI), a micro-software programme has been developed. ETI has been derived from known ELISA O.D. and known reliable API by the formula 270.55 x AR1 ELISA O.D.+7.4 (Table 10).

These three sites were stratified serologically using AR1 ELISA O.D. and ETI values according to which site C had the lowest transmission, site B had the highest and site A had moderate transmission. There is a great need of an alternative surveillance system which can determine low or non-endemic areas since low transmission is difficult to assess microscopically which is simple to determine by serology.

Table 10. Coded blind samples for serological tests

Code No.	Sample size	Mean O.D.±S.D.		API (2000)	ETI
		R1	*Pf*		
R	45	1.2 ±0.273	0.81 ± 0.28	74	334.76
BR	50	0.727 ±0.604	0.77 ± 0.59	48.5	204.08
KB	48	0.76 ± 0.13	0.90 ± 0.14	23.2	213.01
KJ	38	1.0 ± 0.25	1.2 ±0.208	77.5	277.95
RK	65	0.83 ± 0.14	0.76 ± 0.11	28.1	231.95
KG	53	0.85 ± 0.18	0.79 ± 0.14	105.4	237.36
C	104	0.54 ± 0.20	0.49 ± 0.13	0	153.50
PR	139	0.26 ± 0.12	0.24 ± 0.10	0	80.44

Code C: Site A (Shahjahanpur area); Codes R, BR, KB, KJ, RK, KG: Site B (Rourkela area); Code PR: Site C (Bangalore area).

Development of a Field Site for Malaria Vaccine Trial (A Collaborative Project with International Centre for Genetic Engineering and Biotechnology, New Delhi— Funded by Department of Biotechnology, Govt. of India)

There are 13 study villages with a total population of 4,221 under Gurundia and Birkera PHCs of Sundergarh district, out of which eight villages with a population of 2,058 are located in deep forests and five villages with a total population of 2,163 are located in a plain area. The study villages are predominantly inhabited by ethnic tribals — Oram, Munda and Khadia etc.

Parasitological Surveys

The longitudinal and cross-sectional parasitological surveys were conducted in all the study villages. The SPR, SfR, *Pf* per cent and annual parasite incidence (API) in the forest villages were 38.1, 30.8, 80.9 and 323.1 respectively, whereas in the plain area villages these were 20.7,13.4, 64.5 and 43.0 respectively. The malaria incidence was more in the younger age groups up to 15 years and the highest incidence was in the 0–5 years age group in the forest area but in the plain area no malaria case was found in the 0–5 years age group and the malaria cases were evenly distributed among the age groups of 5–10, 10–15 and >15 years.

Out of the total malaria cases in the forest area, the prevalence of *P. falaciparum*, *P. vivax and P. malariae* accounted for 82, 16 and 2 per cent respectively but in the plain area, the prevalence of *Pf* and *Pv* were 68 and 32 per cent respectively. The spleen rate in children living in the forest area villages was above 75 per cent throughout the year and in adults it was above 40% whereas, in the plain area the spleen rate in children and adults ranged from 40–82 and 9–14% respectively.

Entomological Surveys

An. culicifacies was widely prevalent in both the areas, whereas *An. fluviatilis* was not found in the plain area. Results of all night mosquito landing collections on human baits showed that *An. fluviatilis* preferred to bite humans and the man landing rate in the forest area was 13.5 bites per person per night. The man landing rate of *An. culicifacies* in the forest and plain areas was 0.3 and 0.5 bites per person per night respectively. The sporozoite rate of *An. culicifacies* and *An. fluviatilis* was found to be 0.70 and 2.82 respectively and entomological inoculation rate (EIR) for these species was calculated as 0.002 and 0.38 infective bites per person per night in the forest area villages, whereas, it was nil in the plain area.

Multiplicity of Infection

Multiplicity of infection is an important marker to get information about the intensity of malaria transmission as well as development of host-immune responses. Forty-nine field collected blood spots from *P. falciparum* positive patients from forested villages were genotyped using MSP 1 (block 2) and MSP 2 (central variable region) by PCR asay. Primers used were gene specific in primary PCR and family specific in the nested PCR condition. Multiplicity of infection among isolates ranged from 1.1 to 3.28. Number of alleles observed were 22 in MSP 1 and 24 in MSP 2. A high proportion of isolates (65–100%) had multiple infectivity with different genotypes of MSP 1 and MSP 2.

Sequence Diversity

The sequence diversity in three malaria vaccine candidates namely MSP 1_{19} (C- terminal 19KD fragment of MSP 1) EBA 175-RII and TRAP was determined in *P. falciparum* isolates collected from forested villages.

Primers were designed covering part of block 16 and entire block 17 of MSP 1 complete N-terminal portion of TRAP and for EBA-175 region F2. Sequencing of 10 field isolates for MSP 1 has shown polymorphism only at 5 amino acid positions. Out of which four were reported earlier by other workers. Rest of the sequence was conserved in all the 10 field isolates. Sequencing of TRAP N- terminal region in field isolates showed polymorphism at 25 sites, and three were reported for the first time.

Sequencing of EBA-F2 region in 16 field isolates has shown polymorphism at 19 amino acid positions. Only five of these polymorphisms were reported between different strains. Study further revealed that a few selected amino acids are targeted for change. This selection may be to maintain non-synonymous polymorphism in EBA region II, thus not affecting the functional aspects. However, important motifs are found to be conserved in all the three vaccine candidates studied.

Immunological Profile

Immunological studies were carried out to study antibody profiles for three antigens (MSP 1, EBA-175 and TRAP). The antibody levels were higher in individuals from forest areas than those residing in the plain area. However, age-wise increase in antibody level was observed both in forest and plain areas.

GIS Database: Village boundaries of plain area villages Balupatra, Chikatmati, Sarala, Mahaliapalli and Mallikpalli were digitized showing landscape features such as highways, village roads, walk ways, rivers, canals, branch canals, water bodies, houses, schools, shops, club, churches, industries, open space, rice fields, etc.

Data Architecture: A three tier GIS database has been generated. First level, village-wise data, which include census information and malaria data. Second is house-wise data, where data of individual house pertaining to house number, number of rooms in the house, type of house – kuchcha or pucca/human dwelling or mixed dwelling, name of the head man, number of persons in the house, their names, age, sex, religion, tribe, income, etc., number of animals in the house and malaria history. Houses have been depicted by square blocks on the village boundary. Third level is 'Personal' level data, person's name, age, sex, marital status, education level, occupation, malaria history up to four malaria episodes have been included. Persons in the houses have been shown by dots. Number of dots in a house (shown by boxes) show number of persons (Fig. 19).

Fig. 19: GIS-based information system consisting of three tier information on village, house and personal level

Out of five plain area villages house-wise data of three villages have been obtained and put in the GIS database. In forest villages data, there were some discrepancies, that are being sorted out.

Functionality of the System

(1) Information of any village/house or person can be retrieved at the click of the mouse within village boundary/ house/ dots respectively on the map.

(2) Using zoom-in facility one can blow up houses and can see number of persons, by assigning different colour to positive and negative cases both for houses or persons, one can see the house-wise malaria spread or in houses how many persons are sick to evaluate the disease scenario (Fig. 19).

(3) Buffer zones can be created around major breeding sites to see the impact.

(4) Malaria epidemiology can be studied both in space and time where change in malaria situation in any village can be correlated to any specific breeding site or the activity in that area to take situation specific control measure.

(5) Per cent composition of any parameter can be easily mapped to review the situation. For example, if one needs to know the per cent parasite composition—*Pv* and *Pf*, instantly situation of the entire area/houses can be known.

Depending upon the requirement database can be tailor-made and so the analysis algorithm to achieve the desired result.

Evidence-based Situation Analysis of Malaria in five Pilot Districts Under Roll Back Malaria (RBM) Initiative [Funded from WHO Country Budget for Biennium 2000-2001]

State of Goa

The population of Goa State is 1.2 million distributed in an area of 3702 km^2. It is divided into two districts (north Goa and south Goa), which are subdivided into eleven tehsils and its capital is Panaji. For malaria control purposes whole Goa comprises of one district, 19 PHCs, 172 subcentres, 360 census villages, 32 malaria clinics. The average population of the PHC is 53,000 and that of sub-centre is 6,800. In urban areas, there are four urban health centres with attached four NFCP units. There are 30 and 90 government and private hospitals respectively, besides there are a large number of private practitioners.

The malaria control programme is an ongoing programme in the state and is being implemented as per the guidelines of the National Anti Malaria Programme (NAMP). Of the two districts, north district is problematic where urban health centre (UHC)

Panaji, PHCs Aldona, Candolim and Corlim are harbouring high malaria endemicity. Margaon is the only UHC in south district showing alarming increase of malaria incidence. Collection, analysis of the data and the maintenance of the information system are inadequate and village/locality-wise data are not available. Case detection is through PCD only. All fever cases are given presumptive treatment and radical treatment is provided to the confirmed malaria cases. Prevention practices include larviciding, use of fish in wells, source reduction, environmental management supported by legislative measures.

Though malaria is restricted to urban areas, urban malaria scheme (UMS) has not yet been implemented. National filaria control (NFC) unit established earlier is deployed for surveillance, GR and control activities without adequate guidelines, scientific and technical support. A post of District Malaria Officer is to be created in both the north and south districts. The municipal committees unlike in other parts of India do not have the responsibility to control the mosquitoes and mosquito-borne diseases. Building byelaws of Goa state are unique in the country but need to be enforced in an effective manner with strong political support. There are several technical, administrative and scientific posts which are vacant for several years resulting deployment of untrained staff and eventually failure of surveillance and preventive measures leading to periodic local and focal outbreaks of malaria associated with developmental activities.

Parasitological survey revealed that *Pf* is the predominant species. The slide positive rate (SPR) and slide falciparum rate (SfR) were 69.8 and 69.8 in ward no. 1 as against 5.4 and 2.7 in ward no. 12, respectively in Panaji City. Monitoring of chloroquine resistance revealed 22.2% early treatment failure (ETF) and 16.6% late treatment failure (LTF) of malaria cases.

Entomological survey revealed the presence of 13 anopheline species in the selected PHC and of these *An. stephensi* is the vector of malaria and breeds profusely in curing tanks at construction sites. The transmission is high at these sites among labourers who live within the construction complex. The man mosquito contact was 10 per bait per night with biting peak between 0100 and 0300 hours. Man hour density (MHD) of *An. stephensi* ranged from 8–10 in model construction site, Panaji. Larval susceptibility test revealed that the larvae of *An. stephensi* are still susceptible to baytex and abate, however, adult mosquitoes were resistant to DDT but susceptible to synthetic pyrethroids. *An. culicifacies* was not encountered in adequate number, therefore, susceptibility tests were not carried out.

Knowledge, aptitude, behaviour and practice (KABP) studies revealed that about 58% people are getting their blood smears examined at malaria clinic run by the Directorate of Health Services, while 46% preferred to get examined their blood smears in hospitals or diagnostic laboratories. Private practitioners are also willing

to participate in the programme provided that the basic facilities for collection of blood smears and results of blood examination are made available to them. Interactive workshops at central and peripheral level were organized for advocacy.

In Goa state, the Public Developmental Authority (PDA), Municipal Committee, University, Medical College, NGOs, Airport Authority of India, Port Authority of India were identified as potential partners under Roll back malaria (RBM) initiative.

District Tumkur, Karnataka

In continuation of earlier work on situation analysis of malaria, second field survey was conducted in the District Tumkur, during August–September 2001 and following field activities were undertaken.

Parasitological studies were carried out by conducting fever survey in six villages of Mathighatta PHC under Taluka C.N. Hally. The adjacent Taluka Hospital at Sira was also visited. Examination of blood smears revealed overall SPR as 24.44, ranging between 8.62 and 60. It may be pointed out that majority of malaria cases were of *P. falciparum* (*Pf* 92.31%), consisting mostly ring stages. Point prevalence study in a primary school revealed that a large number of students were infected with *P. falciparum* showing lack of typical clinical symptoms of malaria. Hence the existence of asymptomatic malaria cases can not be ruled out in this area.

The team visited two PHCs of Taluka C.N. Hally—C.N. Hally and Mathigatta, and PHC Sira (Taluka Sira). Blood smears collected from patients who reported at PHC hospital in the months of August and September 2001 were cross-checked for malaria parasites. It was observed that quality of blood smear and staining was very poor. Examination of blood smears revealed discrepancy in results particularly false negativity of *P. falciparum* cases by the PHC technicians. It was observed that technicians were capable of identifying *P. falciparum* gametocytes only, while ring stage of *P. falciparum* parasite were invariably missed. Out of 300 confirmed negative slides, 12 were found positive for *P. falciparum* rings only. Altogether *P. falciparum* rings were found to be missed in 23 slides. In order to find out the susceptibility status of *P. falciparum* against chloroquine, 7-day *in vivo* drug sensitivity test was conducted in 19 *P. falciparum* cases. The results of *in vivo* test suggested that chloroquine is highly effective and should be used as a first line of treatment.

It may be mentioned that majority of patients are invariably treated either with sulphadoxine-pyrimethamine combination or E-mal (α and β artether) as a first line of treatment, as evidenced by discussion with private medical practitioners, community, chemists and pharmacists. Various medical stores were visited to know the common antimalarial drugs availability, which revealed that antimalarials other than chloroquine were being used by the patients either by prescription of general

practitioners or by self-medication. The E-mal and ablaquine (chloroquine + bulaquine) were the most commonly used drugs available with medical stores.

Entomological survey was also carried out by conducting indoor resting collection of mosquitoes in few villages of PHCs Mathighatta, C.N. Hally and Dasudi. Six anopheline species—*An. culicifacies*, *An. fluviatilis*, *An. subpictus*, *An. annularis*, *An. pallidus* and *An. vagus* were encountered. Of these, *An. culicifacies* and *An. fluviatilis* are the established vectors of malaria.

Further 33 ovaries of *An. culicifacies* were collected to find out the sibling species composition. Out of twenty-five specimens that could be identified, 22 were species A and three were species B. Beside adult collection larval breeding survey was also conducted in limited mosquito breeding sites—tanks, ponds and wells. Majority of these water habitats were found to be positive for anopheline breeding. It was suggested that in all perennial water bodies larvivorous fishes should be introduced on priority basis.

Two workshops were organized to identify partners to be involved in malaria surveillance, early detection and prompt treatment (EDPT). One workshop was held at district headquarters, Tumkur on 3 September 2001, another at Taluka C.N. Hally of District Tumkur on 7 September 2001. Representatives of various non-government organizations, government and private sectors and community attended the workshops. The target sectors were Health Department, Non-government Voluntary Organizations (NGOs), Private Health Care Providers, Non-health Government Sectors, Education Departments and Community Representatives—MP, MLA, Village Panchayat Head, etc. The objectives of these workshops were to interact with different groups and to get their opinion for their active partnership in formulating malaria action plan.

Based on the findings of two field visits a final project report was prepared and handed over to the authorities of the National Anti Malaria Programme, State Health Department, WHO-SEARO, New Delhi and ICMR.

District Keonjhar, Orissa

The situation analysis of malaria in District Keonjhar, Orissa was carriedout through intensive surveys during September 2001. The studies revealed that all 13 PHCs are highly endemic for malaria. Factors like hilly forested terrain with predominantly tribal population, reservoirs of malaria parasites, presence of highly anthropophagic malaria vector, *An. fluviatilis* and highly inadequate indoor residual spray with DDT contribute for almost perennial transmission of *P. falciparum* malaria. Percentage of *P. falciparum* is consistently over 90% for the past several years with 10 to 30 deaths due to malaria but since last three years the number of malaria deaths had increased

to over 70 each year (108 in 2000). ABER is above 19% since last ten years. Slide positivity rate is above 10% since 1986. Highest API was recorded in 1991, 1992 and 1996 (about 47). Presumptive radical treatment (PRT) is being practiced. District Malaria Officer works under Chief District Medical Officer (CDMO). Most of the posts are filled up except AMO and MPHWs. The provision of adequate financial assistance through EMCP with World Bank assistance is existing. However, due to cumbersome procedure of release, adequate funds are not available in time to DMO.

Procurement and distribution of drugs and laboratory supplies are satisfactory but same is not true for insecticides. Required quantities of insecticides are not projected as per technical requirement due to inadequate operational cost. Problems have also been faced for warehousing of insecticide. Transport is not adequate. Supervisory and monitoring staff is required. Accessibility to remote villages is difficult resulting in poor surveillance and difficulty in referral of severe cases. There are a large number of private practitioners, but they do not follow the national drug policy. *In vivo* study of therapeutic efficacy of antimalarials in two PHCs revealed that *P. falciparum* is susceptible to chloroquine. *An. fluviatilis* species S was incriminated and found to be the principal vector of malaria. Susceptibility status of vectors is not being monitored regularly. Investigations revealed that *An. fluviatilis* is highly susceptible to DDT, malathion and deltamethrin. However, *An. culicifacies* was found to be resistant to DDT. Deltamethrin treated mosquito nets are being used in one PHC under EMCP.

KABP studies revealed the awareness about malaria transmission and its proper treatment was much less in tribal population as compared to non-tribals. Many of the tribals go to spiritual healers and use primitive traditional methods of personal protection. Tribal population are also reluctant to get their blood examined at the onset of fever and they do not get their houses sprayed with insecticide. They need to be given proper health education pertaining to the above mentioned points

Two workshops were conducted at district and PHC level. Interactions with officers belonging to health, non-health departments and NGOs revealed that there is ample scope of establishing partnerships and link with them to combat malaria under RBM initiative. The response from non-health departments, private sectors and other organizations was very encouraging and they offered their services in various malaria control activities to be undertaken under RBM initiative.

District Aizawl, Mizoram

A field visit was undertaken in Aizawl, Mizoram in September 2001 to collect evidence-based research data for situation of malaria in Aizawl (West), Mizoram.

Parasitological survey was carried out in seven villages of Kolasib, Sairang and Lengpui PHCs. In addition, blood slides were collected from OPD at Kolasib. Of

827 slides collected, only 19 were positive (8 *P. vivax* and 11 *P. falciparum*) giving SPR 2.29, SfR 1.3 and *Pf* % 57.8. Of 11 *Pf* cases only five could be followed for seven days *in vivo* chloroquine sensitivity test. Of five subjects four were found fully susceptible, while one case showed parasitaemia on Day 3 but it was cleared on Day 5.

Entomological surveys for prevalent breeding habitats of anopheline mosquitoes and adult mosquito density in houses were carried out in all seven villages. Collection of landing mosquitoes on human baits and light-trap collection outdoors for four nights was also undertaken. Light-trap collection and night bait collection did not yield any anopheline mosquitoes. Only *An. vagus* and *An. barbirostris* were collected from indoor resting sites. Two *An. dirus* mosquitoes emerged from the larvae collected from water collection in rock crevices. Rice fields and other water bodies yielded *An. vagus* and *An. barbirostris. An. minimus* was not encountered.

Health seeking behaviour of communitiy was also assessed through questionnaires in Rengtakawn, Meidum, Lengpui and Sairang villages. Preliminary findings indicate that community is well aware of the use of mosquito nets, they go to the health centres when fallen sick but do not comply to complete treatment.

In order to create awareness, sensitization of the different sectors and communities and to seek their participation in malaria control two workshops were organized—one at Aizawl (for district level) and another at Kolasib (for community level workers). The workshop at Aizawl was inaugurated by the Hon'ble Mininster of Health, Govt. of Mizoram and was attended by 23 participants including four NGOs and Medical Officers. District Commissioner, Aizawl agreed to coordinate different sectors for malaria control as per the need of local health department. The workshop at Kolasib was attended by 38 participants including, D.C., Medical Officers, YMA, MHIP, MUP, teachers, pastors, journalists, village council chiefs, etc. There has been headway in forming district (recently constituted) level committee of different sectors for malaria control.

District Jodhpur, Rajasthan

District Jodhpur in the western region of Rajasthan was one of the five pilot districts selected for implementation of RBM initiative. An initial survey was carried out in November 2000 to collect data/information on infrastructure of Health Care System, resources and retarding factors for effective malaria control. Later, a study was carried out in September 2001 to ascertain the factual validity of some key factors (mainly operational).

Out of 10 Community Health Centres (CHCs) in the district, two CHCs with high and low API were selected on the basis of last five years epidemiological data provided

by NAMP for evidence-based situation analysis. Further in each of the selected CHCs, two villages with low and high API were selected for the studies and to understand the health seeking behaviour of malaria patients and to study KABP among local community a survey was carried out in villages using a structured questionnaire. Data and information collected on different aspects pertaining to malaria control programme were analyzed and suitable suggestions were made.

Epidemiological data of the district for the past 10 years (1991–2000) revealed API in the range of 0.3–6.2, SPR 0.3–6.3 and *Pf* per cent 6–65, respectively. Of the total 512 subcentres in the nine CHC areas, high risk areas of the district were stratified based on API and *Pf* % data of three years (1997–1999). A total of 46 subcentres were having API between 2 and 5, 16 between 5 and 10 and 11 above 10. *P. falciparum* rate was above 30% in 42 subcentres.

For entomological studies seven villages were selected in two CHCs. Two malaria vectors, *An. stephensi* and *An. culicifacies* were prevalent in both CHCs. Density of adult *An. culicifacies* was maximum in stone quarry areas of CHC Banar. Anopheline breeding habitats (per cent positive) were also maximum in stone quarry areas. *An. stephensi*, the major vector of malaria was found breeding extensively in underground water storage tanks which can be made mosquito proof by simple engineering methods—wire mesh. In District Jodhpur selective indoor residual spray is being done with DDT. In the present survey, prevalent vector species namely *An. stephensi* and *An. culicifacies* were found resistant to DDT in two high risk PHCs, Banar and Fidusar.

Two advocacy workshops on intersectoral coordination among potential partners for control of malaria under RBM initiative were organized. Participants were invited from different sectors of Government, non-Government organizations and private sectors. Students and community leaders also participated in the district level workshops. A total of 40 participants attended the workshop. The participants were appraised of the concept of RBM initiative for malaria control. They were briefed about the objectives of RBM and their role as individuals and as a part of their organizations in the management of malaria through partnership.

The study revealed that most of the private medical practitioners do not follow radical treatment of malaria (with primaquine) to interrupt further transmission of malaria in the community. Therefore, NGOs involved in health activities, private medical practitioners, nursing homes and other health providers should be made aware of the national drug policy and treatment of malaria cases and specially of complicated cases. These agencies should be sensitized to extend cooperation to Government Health Services. Paramedical staff with these private agencies may also be given adequate technical training to support health system.

One of the main reasons for persistence of malaria in few areas could be due to inadequate surveillance and treatment mainly because of inaccessibility and tough

terrain. In such a situation, malaria patients visiting the nearby private practitioner (mostly quacks) are administered suppressive treatment by intramuscular injection of chloroquine (40–80 mg base) resulting in administration of subcurative doses.

From the KABP study done, it was observed that the people have fairly good knowledge about malaria, its treatment and control etc. People in general are aware of personal protectoin measures, but most of them are not actually using these methods due to economic reason or variation in the attitude. Therefore, is advisable to motivate the community through IEC activities.

Highlight of the Research Activities Carried Out by MRC Field Stations under the Integrated Vector Control of Malaria Project

Nadiad (Gujarat): Urban malaria projects in Surat and Ahmedabad were completed, and technology transferred. In collaboration with WHO, new insecticides are under field evaluation. Field evaluation of nets treated with bifenthrin and a new formulation of deltamethrin was undertaken. A study of epidemiological efficacy of malathion indoor residual spraying was initiated. Work on the health impact assessment of Sardar Sarovar project was initiated. Epidemic containment support in earthquake-affected areas was provided.

Jabalpur (Madhya Pradesh): Investigations on malaria outbreak in Betul district was carried out. Field work on clinical trial of chloroquine + azithromycin was completed. Paracheck diagnostic test kit was evaluated in the field. Tolerability and efficacy of artesunate + chloroquine or sulphapyremethamine combination vs. single agent chloroquine or sulphapyremethamine for uncomplicated falciparum malaria were evaluated. Study on anopheline ecology and malaria prevalence in villages near Bargi Dam was done. Follow-up investigation on malaria outbreak in the villages of Narayanganj PHC, Mandla was carried out. Evaluation of the impact of DDT indoor residual spraying was conducted in four villages of Chhindwara district.

Hardwar (Uttaranchal): Work on new antimalarial drug development (reaction product of sulphadoxine) was carried out. One of the compounds from a plant showed good antiparasitic activity against *Trypanosoma brucei brucei* (WHO results), besides having antimalarial activities. Concentrations of allethrin released by mats in air were found higher in room with air cooler than in those with open windows without cooler. Follow-up work of the impact of bioenvironmental control methods in industrial complexes was continued. Parasitological and entomological surveys were carried out in Laksar PHC of District Hardwar. A project on evaluation of the impact of malathion indoor residual spraying being used in malaria control was also completed.

Sonapur (Assam): Clinical trial of combination therapy of chloroquine + azithro-

mycin against *P. falciparum* was completed. Multidrug resistance study on *Pf* malaria in Sonapur was carried out.

Chennai (Tamil Nadu): Role of ecological variants of *Anopheles stephensi* in malaria transmission was studied. Association of rainwater harvesting devices and vector breeding was studied. Field evaluation of hilmilin, an insect growth regulator, against culicines was undertaken. Health education and training programmes were also undertaken. Malaria clinic was run for prompt diagnosis and early treatment.

Rourkela (Orissa): Longitudinal and cross-sectional parasitological and entomological studies were conducted to develop suitable sites for malaria vaccine trials. Epidemiological studies were conducted to understand the role of *An. culicifacies* and *An. fluviatilis* sibling species in malaria transmission dynamics and to characterize parasite population and immune response in a tribal population. Studies on host feeding preferences and susceptibility status of malaria vector species were carried out. Gametocytocidal efficacy of compound 80/53 in uncomplicated *P. falciparum* cases was evaluated. The prevalence of haemoglobinopathies in different tribal communities was studied.

Haldwani (Uttaranchal): Malariogenic stratification to identify malaria risk factors was carried out in two PHCs in Terai/Bhabar areas. Larvicidal evaluation of *Bacillus thuringiensis* var. *israelensis* was carried out.

Car Nicobar (A&N Islands): Epidemiological investigation of malaria in creek and non-creek areas was carried out. Malaria prevalence among Jarawas, a primitive tribe was studied.

Panaji (Goa): Three indigenous mosquito pathogenic *Bacilli* were isolated, characterized and tested. A malaria outbreak in Sanguem PHC was investigated. Ecology and bionomics of *An. stephensi* in urban ecosystem were studied. Rapid diagnostic kits were tested for NAMP. A field trial of *Bti* was carried out. Transfer of bioenvironmental control technology to Mormugao Port Authorities continued in contract research mode.

Bangalore (Karnataka): Bioenvironmental control strategy was extended in Mangalore city by intersectoral participation and NGO support. Follow-up of this strategy in Kolar and Hassan districts was carried out and the Govt. of Karnataka has drafted a plan to implement this strategy in the whole state. Work on development of mosquito control plan in Bangalore city has been started. An evidence-based malaria situation analysis in District Tumkur under RBM initiative was carried out. A study on the remote sensing for delineation of breeding habitats of *An. culicifacies* was also carried out. Evaluation of the impact of DDT indoor residual spraying was done in District Mandya.

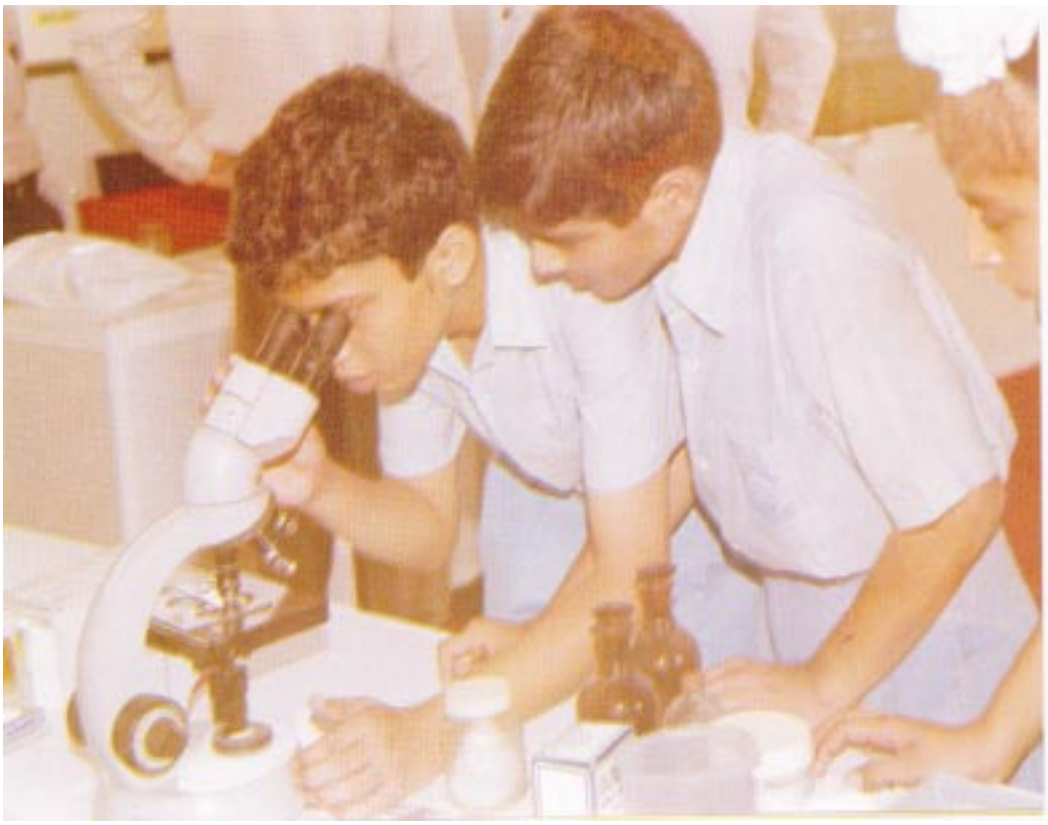
Shahjahanpur (U.P.): Geographical reconnaissance of larval habitats in Shahjahanpur was continued. Field evaluation of the larvicidal efficacy of *Bti* was carried out.

Shankargarh (U.P.): Diagnostic and treatment services were continued through malaria clinic as a passive surveillance agency to monitor disease prevalence in the area.

INFORMATION, EDUCATION AND COMMUNICATION (IEC)

National Science Day Celebrations

National Science Day (NSD) was celebrated at a public school in Delhi during the last week of February. The theme of the NSD for the year 2001 was "Information Technology for Education". Health education activities like speeches, video-film show, slides show, exhibition and live demonstrations were carried out. Students were briefed about malaria and its preventive and control measures. Pamphlets about malaria control measures were distributed. Some informative books and posters were given for school library.



School children observing malaria parasites under the microscope

Observance of Antimalaria Month

Health education camp was organized during antimalaria month in Bal Bhavan – A government vocational training centre for children. The activities conducted by the Malaria Research Centre for the children included lectures in non-technical language, live demonstrations, video films, slides show, exhibition on different aspects of malaria.

Health Camp at a Private Organization

A health camp was organized at Divine United Organization, Shahur, Mehrauli, Delhi in the last quarter of 2001. Over 200 health workers from different states of India participated in this health camp. They were briefed about mosquito and malaria control activities. Live demonstration on mosquito life-cycle and use of larvivorous fish was done.

Video Film Produced

A film on Konkan Railways entitled, "Konkan railways – A boon or a bane" was completed after shooting in Goa, Karnataka and Maharashtra. This film highlights the health impact assessment of the Konkan railway developmental project.

Distribution of VHS Cassettes

About 160 video cassettes were copied and 47 VHS cassettes on different aspects of malaria were sold/distributed to the trainees, different departments and NGOs.

Still Photography

During the period under report over 25 print and slide film rolls were exposed and printed. The work involved still photography of Centre's scientific activities, meetings, trainings and other functions.

BIOLOGICAL MATERIAL BEING MAINTAINED AT THE CENTRE

Mosquito Species

An. stephensi

From urban and semi-urban areas

Nehru Place, Delhi
Okhla, Delhi
Chennai, Tamil Nadu

From rural areas

Ladpur, Haryana

Morphological mutants

Red eye (r)	–	sex linked recessive
Black larvae (bl)	–	autosomal semi-dominant
Golden yellow (gy)	–	autosomal recessive
Creamish white eye (cw)	–	new mutant
Reddish brown eye (rb)	–	new mutant

Biochemical variants

Bahadurgarh (EST-2)

Insecticide resistant lines

Malathion resistant
Permethrin resistant
Lambdacyhalothrin resistant
Deltamethrin resistant
Cyfluthrin resistant
Fenthion resistant

An. culicifacies complex

Species A

Dehra, Uttar Pradesh
Burari, Delhi
Rourkela, Orissa

Species B

Acrocentric Y-chromosome lines

Ladpur, Haryana
Haldwani, Uttar Pradesh

Submetacentric Y-chromosome lines

Rameshwaram, Tamil Nadu
Rourkela, Orissa

Insecticide resistant lines

DDT resistant – Ladpur, Haryana
Malathion resistant – Ladpur, Haryana

Species C

Submetacentric Y-chromosome line

Jabalpur, Madhya Pradesh

Insecticide resistant line

DDT resistant – Jabalpur, Madhya Pradesh

***An. fluviatilis* complex**

Species S and T – Rourkela, Orissa
Species T and U – Hardwar, Uttaranchal
Species T – Haldwani, Uttaranchal

An. sundaicus

Cyclic colonies established from Car Nicobar
Katchal, Tressa (A & N Islands)

An. annularis

Nathupura, Delhi

Aedes aegypti

Delhi

Culex quinquefasciatus

Delhi
Sonepat, Haryana
Mewat, Haryana

Insecticide resistant lines

Malathion resistant	–	Sonepat, Haryana
Permethrin resistant	–	Sonepat, Haryana
Lambdacyhalothrin resistant	–	Sonepat, Haryana
Deltamethrin resistant	–	Sonepat, Haryana
Cyfluthrin resistant	–	Sonepat, Haryana
Fenthion resistant	–	Sonepat, Haryana

Morphological mutants

Red eye (re)
Scarlet eye (se)

Biological Material Available at the Parasite Bank

Human Plasmodia

P. falciparum

Non-adapted cryopreserved isolates

Non-adapted field isolates having different cytoadherence and erythrocyte invasion properties

Adapted cryopreserved isolates

Sera/plasma from infected patients

Different stages of the parasite from culture

- Merozoites (from culture supernatant)
- Ring (by synchronization)
- Gametocytes (by Hypoxanthine treatment)
- Free parasites for antigen preparation (by Saponin lysis and ultrasonication)

P. vivax

Non-adapted cryopreserved isolates
Sera/plasma from the infected blood
Sporozoites harvested from artificially fed mosquitoes

P. malariae

Non-adapted cryopreserved isolates
Plasma from the infected blood

Non-human Plasmodia

Different species of avian, simian and rodent plasmodia
Rodent plasmodia infected rats/mice
Sera/plasma from respective vertebrate hosts

Cell Lines

- Hepatoma cell line: Hep G2 A16 used in the *in vitro* cultivation of exo-erythrocytic stage malaria parasites
- Myeloma cell line: SP2
- Hybridomas: 2A 10 (anti-*P. falciparum* sporozoite antibody secreting cells)
- 2 F2 1 A7 (anti-*P. vivax* sporozoite antibody secreting cells)

Non-human malaria parasites available at the Parasite Bank

Parasite species	Source	Susceptibility to antimalarials
Simian malaria		
P. cynomolgi bastianelli	NICD, Delhi	Not done
P. knowlesi	–do–	–do–
P. fragile	CDRI, Lucknow	–do–
Avian malaria		
P. gallinaceum	NICD, Delhi	Not done
P. relictum	Wild, Delhi	–do–
Rodent malaria		
P. berghei NK-65	PGI, Chandigarh	–do–
P. berghei NK-65*+	CDRI, Lucknow	CQ sensitive
P. berghei *	–do–	CQ resistant
P. berghei	–do–	Quinine resistant
P. chabaudi	INSERM, Paris	Not done
P. vinckei petteri 279 BY	–do–	–do–
P. yoelii yoelii 265 BY**	–do–	–do–
*P. yoelii nigeriensis***+	LSHTM, London	–do–
P. yoelii nigeriensis	CDRI, Lucknow	Multi resistant
P. yoelii	ICGEB, New Delhi	Not done

*Oocyst positive in *An. stephensi*; **Oocyst and sporozoite positive in *An. stephensi*; +Infective gametocyte producing strain.

Details of *P. falciparum* isolates collected and adapted *in vitro*

Place of collection	No. of isolates collected	Adapted/ Cryopreserved*
Delhi	172	70
Ghaziabad (Uttar Pradesh)	27	22
Shankargarh (Uttar Pradesh)	39	27
Baharaich (Uttar Pradesh)	21	–
Gautam Budh Nagar (Uttar Pradesh)	39	–
Shahjahanpur (Uttar Pradesh)	6	6
Mandla (Madhya Pradesh)	23	15
Jagdalpur (Madhya Pradesh)	14	6
Sonapur (Assam)	25	2
Rourkela (Orissa)	33	9
Rameshwaram (Tamil Nadu)	1	1
Jaisalmer (Rajasthan)	39	27
Bharatpur (Rajasthan)	35	1
Alwar (Rajasthan)	25	–
Nuh (Haryana)	25	2
Kolkata (West Bengal)	19	
Visakhapatanam (Andhra Pradesh)	12	–
Collected during 2001		
Delhi	3	
Bissam Cuttack (Orissa)	22	
Total	580	188

*****Continuous cultivation and adaptation was discontinued due to the shortage of normal human blood and serum.

Details of adapted/characterized *P. falciparum* parasites

Species/Strains of parasite	No. of isolates
Adapted isolates susceptible to chloroquine	54
Adapted isolates resistant to chloroquine	52
Adapted isolates to be tested for their sensitivity to chloroquine	82
NF-54, an infective gametocyte producing strain of *P. falciparum*	1
3D 7A : a clone of NF-54	1
A-4 : a clone with binding property to CD36	1
Dd2: a clone which can invade trypsin treated erythrocytes	1
Field isolates which can invade trypsin treated erythrocytes	3
Field isolates which can invade neuraminidase treated but not trypsin treated erythrocytes	3
Field isolates which can invade normal erythrocytes but not in neuraminidase or in trypsin treated erythrocytes	3
Field isolates which can invade both in neuraminidase treated and in trypsin treated erythrocytes	5
Field isolates which can form rosettes	3
Field isolates which can bind to CSA	1
Field isolates which can bind to CD36	9
Field isolates which can bind to ICAM-1	2

Experimental Animal Facility

Rabbits, pigeons, domestic fowls, laboratory mice, etc. were procured, maintained and utilized for research purpose throughout the year as per the guidelines issued by the concerned authorities. These animals were housed at 22, Sham Nath Marg and 2, Nanak Enclave buildings and were used as blood meal source to mosquitoes of different species and strains maintained at the Centre. Animals that fell ill in the process of feeding the mosquitoes were given treatment and rest as and when required. Laboratory mice were used in screening the antimalarials, host-parasite intraction studies and maintenance of rodent plasmodia at the parasite bank. Carcases were disposed properly. Experiments on animals were performed with the approval of SAC and Institutional Animal Ethics Committee (IAEC) of the Centre. The IAEC meetings were conducted and approval for the seven proposals regarding use of animals in maintaining biological materials and research experiments was taken from the Chairman of the IAEC after discussion. Proposal for renovation/upgradation

of the existing experimental animal facilities along with budget estimate was sent to the Director-General, ICMR for necessary action. Requirements and suggestions were given regarding animal testing facility which is to be constructed as a part of Research Block of MRC building at Dwaraka.

TRAININGS/WORKSHOPS ORGANIZED BY THE CENTRE

Comprehensive Vector Control (CVC) Training Course

A six-week training course sponsored by the NAMP for Entomologists/Biologists working in different states was organized from 13 February–27 March. A total of 25 participants attended the course. Training course was on modular pattern-based on interactive learning. Regular lectures by eminent scientists were followed by the group exercises and presentations made by the participants. In addition to practical sessions, participants were taken to Alwar (Rajasthan) and Haldwani (Uttaranchal) for field exercises in CVC. Panel and group discussions were also held. Pre- and post-tests were conducted to assess the knowledge gained through training. Participants presented their tour report at the end of the training session. On the last day certificate of participation was distributed to all the trainees at the valedictory function. (Course Director : Dr. Sarala K. Subbarao; Course coordinators : Dr. C.P. Batra, Dr. Nutan Nanda, Dr. B.N. Nagpal from MRC and Dr. R.S. Sharma from NAMP, Delhi)

Participants and faculty members of CVC training course

Workshop to Develop Common Working Protocols for Situation Analysis of Malaria in Five Pilot Districts under Roll Back Malaria (RBM) Initiative

The workshop was organized by Malaria Research Centre, Delhi at NICD from 23–25 May 2001. There were 23 participants, 8 from state governments (Goa, Karnataka, Rajasthan, Orissa and Mizoram), 5 from MRC field stations and 10 from MRC, Delhi, besides the resource persons and faculty members. Dr. S. Pattanayak, ex-Director, NAMP, Dr. P.R. Arbani, Sr. Advisor, WHO-SEARO, Dr. V.P. Sharma, Consultant, RBM, WHO-SEARO, Dr. S. Phukan, Addl. Director, NAMP and Dr. S.K. Subbarao, Director, MRC attended the inaugural session where the need of

Participants of Roll Back Malaria Workshop

evidence-based research and involving nonhealth departments, NGOs, women organizations, community, etc. as potential partners in malaria control activities was emphasized. The technical sessions included lectures by Shri. N.L. Kalra, Consultant, MRC, Dr. Prema Devraj, Representative, NGO, Dr. V.K. Monga, President, Delhi Medical Association, Dr. Alpana Sagar from JNU and Dr. Padam Singh, Addl. DG, ICMR on various topics like situation specific strategies of malaria control, factors influencing health seeking behaviour of the communities, statistical basis of survey design and estimation of sample size etc. The participants were divided into five groups as per selected sites. All concerned scientists from MRC and counterparts from respective districts/states discussed about the activities to be undertaken for evidence-based survey and identified the parameters to be studied for knowing the transmission dynamics in respective areas. Presentations were made by each group about the plan of activities to be undertaken in each selected site for RBM initiative. After presentations, formats for collection of entomological, parasitological and sociological data were discussed by the resource persons and participants. Finally, common working protocols were developed.

Workshop on Therapeutic Efficacy of Antimalarials

The workshop was organized at MRC on 8 November and the scientists from MRC and field stations attended the workshop. Procedures for monitoring resistance to antimalarials using new therapeutic efficacy protocols were presented and discussed. Dr. V.K. Dua and Dr. Hema Joshi presented the methods of sample collection for molecular biology of drug estimation studies.

Workshop on Determination of Insecticide Resistance:Methods and Modalities

The workshop was organized at MRC on 8 November and scientists from Malaria

Research Centre, Delhi and officer-in-charge of all the MRC field stations attended the workshop. Lecture-cum-demonstration on the above topic was held. The scientists were appraised of the techniques for determination of resistance in field populations of *Anopheles* vectors. Different aspects such as collection of mosquitoes, sampling techniques, transport and exposure of mosquitoes to insecticide treated papers and interpretation of the data etc. were discussed. They were provided with necessary protocol and working notes.

CAPACITY BUILDING

1. Dhiman, R.C. Participated in WHO sponsored workshop on Epidemic Preparedness and Response held at National Insitute of Epidemiology, Chennai from 26 November to 6 Decembser.

2. Ghosh, S.K. Awarded a WHO Fellowship and he attended a two-week 'International Communicable Diseases Course' at the Ministry of Public Health, Thailand from 3-14 September.

3. Joshi, Hema. Attended a training course on "Biosocial Research Methodology" at Institute for Research in Medical Statistics, New Delhi from 26 February to 3 March..

4. Reetha, A.M. Attended a training course on Bio-social Research Methodology in Statistics at Institute of Research for Medical Statistics 26 Feb to 3 March.

5. Shahi, B. Participated in WHO sponsored Workshop on Epidemic Preparedness and Reponse held at National Insitute of Epidemiology, Chennai from 26 November to 6 December.

6. Valecha, Neena. Attended a training course on Bio-social Research Methodology in Statistics at Institute of Research for Medical Statistics from 26 Febrery to 3 March.

HONOURS AND AWARDS

1. The H. E. Governor of Goa nominated Dr. Ashwani Kumar, OIC, MRC Field Station, Goa on the Governing body of the Indian Red Cross Society, Goa Branch. He was also nominated on the Governing Board of the Goa Health Collective as Malaria Expert.

2. Dr. S. K. Sharma, MRC Field Station, Rourkela was honoured by Rotary Club of Rourkela (Central) on July 7 for outstanding efforts on Rotary Against Malaria.

3. Dr. V. K. DUA, MRC Field Station, Hardwar was awarded Dr. M.O.T. Iyengar Memorial Award for the year 1999 of ICMR.