1

Vector Biology and Control

1.1 Vector biology

1.1.1 Computerised key of mosquitoes of India

The information on taxonomy, distribution and biology (breeding habits, biting time, resting habits, etc.) of 270 species of mosquitoes belonging to genus *Anopheles, Culex, Aedes, Mansonia, Armigeres, Toxorhynchites* and *Orthopodomyia* which has been extracted out from the literature is being computerised. Brief biology and distribution maps of 170 species have been completed. Collection of information for rest of the species is in progress.

1.1.2 *Anopheles culicifacies* Complex

Bionomics and distribution pattern

An. culicifaies populations from Chandel, Sarlya, Chikdalyia, Tikri Ghat and Gujar Kheri villages in Khandwa district (Madhya Pradesh) were analysed for sibling species composition and host preference. Results revealed that species A and C, the established vectors of malaria comprised of > 75% of the total *An. culicifacies* population thereby indicating high malariogenic potential of the study villages. A longitudinal study on the bionomics of *An. culicifacies* sibling species in Dindori and Mandla districts (Madhya Pradesh) was continued for the second successive year in collaboration with the Regional Medical Research Centre for Tribals, Jabalpur. Observations revealed predominance of species C (> 80%) in the study villages in all the seasons and confirmed that this species is playing a major role in malaria transmission in both the districts. In all the above mentioned districts, analysis of blood meal source of *An. culicifacies* sibling species using counter current immunoelectrophoresis revealed these to be primarily zoophagic.

1.1.3 *Anopheles fluviatilis* Complex

Distribution, bionomics and biology of sibling species

A longitudinal study on the bionomics of *An. fluviatilis* species V in malarious Laksar PHC of Hardwar district was completed. Results revealed that although species V comprised 20.3% of the total *An. fluviatilis* population, but majority of it (77%) preferred to rest in human and mixed dwellings (Fig. 1.1). The overall anthropophilic index (AI) of species V was 4.13% (Fig. 1.2) whereas the other sympatric species, *An. fluviatilis* species T and U and

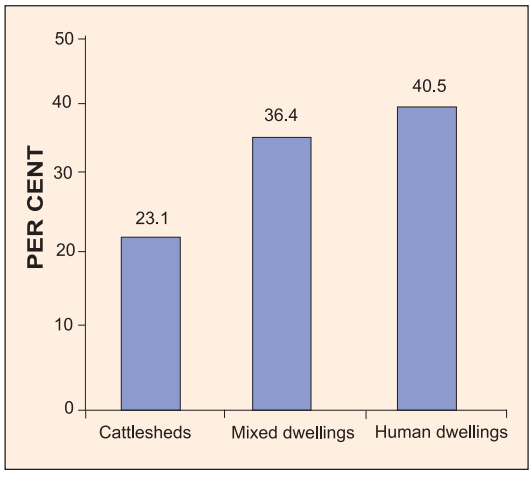

Fig. 1.1: Relative proportion of *An. fluviatilis* species V resting in different types of dwellings in study villages of Laksar PHC (Hardwar)

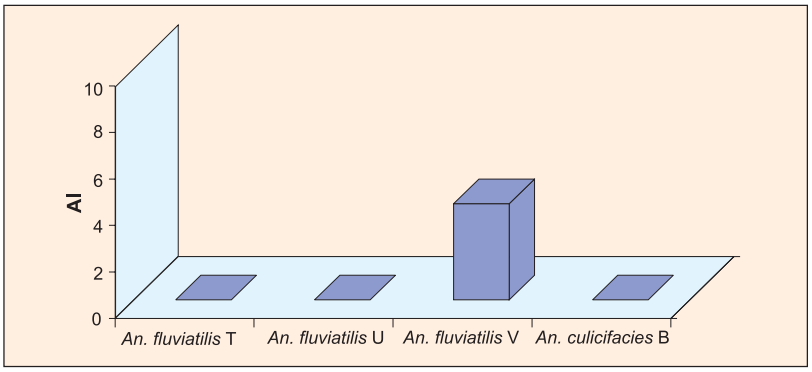

Fig. 1.2: Anthropophilic index (AI) of sibling species in study villages of Laksar PHC (Hardwar)

An. culicifacies species B were found to be totally zoophagic. The proportion of species V was more in the months of October–December when majority of malaria cases were encountered. These observations strongly suggest that species V is playing an important role in malaria transmission in Laksar PHC.

Laboratory rearing experiments were carried out using single female cultures of *An. fluviatilis* species T, U and V. Parameters like number of eggs laid per female, percent hatchability, duration of larval and pupal stages and survival of adult males and females were recorded for each replicate. Observations revealed no significant difference in fecundity, duration of aquatic phase of development and longevity of adults of these sibling species.

Efforts to colonise *An. fluviatilis* species U were successful which is being maintained as a cyclic colony. A study has been initiated to resolve the taxonomic status of members of the Fluviatilis-Minimus group using cytological and molecular tools. Ovaries of *An. minimus* collected from north-eastern states have been processed for polytene chromosome preparations. A photomap of polytene chromosomes complement of *An. minimus* would be prepared and compared with that of *An. fluviatilis* for cladistic analysis.

Taxonomic status of *An. fluviatilis* S as evidenced by molecular characterisation

In a recent taxonomic update by Harbach[1], *An. fluviatilis* S has been regarded as synonym with *An. minimus* species C and thus the later being senior synonym replaced *An. fluviatilis* S. Consequently, he considered the Fluviatilis Complex comprising of two species only—species T and U. Several other recent publications also considered *An. fluviatilis* S as synonym with *An. minimus* species C[2-4]. These reports are primarily based on homology in 335 base pair nucleotide sequence of D3 domain of 28S ribosomal DNA (rDNA) of *An. fluviatilis* S reported by Singh *et al*[5] with that of *An. minimus* species C. In order to resolve the taxonomic status of *An. fluviatilis* S, we determined the nucleotide sequences of a mitochondrial gene cytochrome oxidase II, (COII) and complete internal transcribed spacer 2 (ITS2) region of rDNA of *An. fluviatilis* S and compared these along with already available 28S-D3 rDNA sequence[5] with other members and of Fluviatilis and Minimus Complexes.

A total of seven specimens of *An. fluviatilis* S collected from Sundargarh (22.1°N, 84.0°E) and Koraput (18.8°N, 82.7°E) districts of Orissa state (India), were sequenced for COII and six for ITS2

[1]**Harbach RE (2004).** *Bull Ent Res*, **95:** 537–553.
[2]**Garros C *et al* (2005).** *J Med Entomol*, **42:** 522–536.
[3]**Garros C *et al* (2006).** *Trop Med Intl Hlth*, **11:** 102–114.
[4]**Chen B *et al* (2006).** *Med Vet Entomol*, **20:** 33-43.
[5]**Singh OP *et al* (2004).** *Am J Trop Med Hyg*, **70:** 27–32.

(a)

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An. fluviatilis S  ATGGCAACAT GAGCAAATTT AGGGCTGCAA GATAGATCAT CTCCTTTAAT AGAACAATTA AACTTTTTTC   70
An. fluviatilis T  .......... .......... .......... .......... .......... .......... ..........   70
An. fluviatilis U  .......... .......... .......... .......... .......... .......... ..........   70
An. minimus C      .......... .......... ...AT..... .......... .A........ .......... ..T.......   70

An. fluviatilis S  ACGATCATAC ATTATTAATT TTAACAATAA TTACAATTTT AGTTGGATAT ATTATAGGAA TATTATTATT  140
An. fluviatilis T  .......... .......... .......... .......... .......... .......... ..........  140
An. fluviatilis U  .......... .......... .......... .......... .......... .......... ..........  140
An. minimus C      .......... .......... .......... .......... .......... .......... ..........  140

An. fluviatilis S  TAATAAATTT ACTAATCGAT ATTTACTTCA TGGACAAACT ATTGAAATTA TTTGAACTGT ATTACCAGCA  210
An. fluviatilis T  .......... .......... .......... .......... .......... .......... ..........  210
An. fluviatilis U  .......... .......... .......... .......... .......... .......... ..........  210
An. minimus C      .......... .......... .......... C......... .......... .......... ..........  210

An. fluviatilis S  ATTATTTTAA TATTTATTGC TTTTCCTTCT TTACGATTAT TATATTTAAT AGACGAAATT AATACACCTT  280
An. fluviatilis T  .......... .......... .......... .......... .......... .......... ..........  280
An. fluviatilis U  .......... .......... .......... .......... .......... .......... ..........  280
An. minimus C      .......... .......... ...C...... .......... ....C..... .......... ..........  280

An. fluviatilis S  CTATTACTTT AAAATCAATT GGTCATCAAT GATATTGAAG TTATGAATAT TCAGATTTTT TAAATTTAGA  350
An. fluviatilis T  .......... .......... .......... .......... .......... .......... ..........  350
An. fluviatilis U  .......... .......... .......... .......... .......... .......... ..........  350
An. minimus C      .......... .......... ..A....... .......... C..C.....C .......... ..........  350

An. fluviatilis S  ATTTGATTCA TATATAATTC CAACAAATGA ACTTGAAACA AATGGATTTC GACTATTAGA TGTTGATAAT  420
An. fluviatilis T  .......... .......... .......... .......... .......... ....G..... ..........  420
An. fluviatilis U  .......... .......... .......... .......... .......... .......... ..........  420
An. minimus C      ......C... .........C. .......C.. .......... ........C. ..T....... ..........  420

An. fluviatilis S  CGAATTGTTT TACCTATAAA TAATCAAATT CGAATTTTAG TTACAGCAAC TGACGTATTA CATTCTTGAA  490
An. fluviatilis T  .......... .......... .......... .......... .......... .......... ..........  490
An. fluviatilis U  .......... .......... .......... .......... .......... .......... ..........  490
An. minimus C      .......... .......... .......... .......... .......... ...T...C.. ..........  490

An. fluviatilis S  CAGTTCCTTC TTTAGGAGTA AAGGTTGATG CAACACCAGG ACGATTAAAT CAAATTAATT TTTTAATTAA  560
An. fluviatilis T  .......... .......... .......... .......... .......... .......... ..........  560
An. fluviatilis U  .......... .......... .......... ....C..... .......... .......... ..........  560
An. minimus C      .......... ......G... .......... .......... ....C..... .......... .......C..  560

An. fluviatilis S  TCGACCAGGA TTATTTTTTG GACAATGTTC AGAAATTTGT GGGGCAAATC ATAGATTTAT ACCAATTGTA  630
An. fluviatilis T  .......... .......... .......... .......... .......... .........C ..........  630
An. fluviatilis U  .......... .......... .......... .......... ..A....... .......... ..........  630
An. minimus C      .......... .......... .......... .......... ..A....... .......... ..........  630

An. fluviatilis S  ATTGAAAGAA TTCCTATAAA TTATTTTATT AAATGAATTA CTTCTATAAC TAATT  685
An. fluviatilis T  .......... .......... .......... .......... .......... .....  685
An. fluviatilis U  .......... .......... .......... .......... .......... .....  685
An. minimus C      .......... .......... .......... .......... .......... .....  685
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(b)

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An. fluviatilis S  AATTCCTTGT TACACAATAT TCTAACTACA TGGCGCCCGT GTACGGACGG CATCATGGCG AGCAGCCCGC   70
An. fluviatilis T  .......... ......C... .......... .......... .......... .......... ..........   70
An. fluviatilis U  .......... ......M... .......... .......... .......... .......... ..........   70
An. minimus C      .......... .......... .......... .......... .......... .......... ..........   70

An. fluviatilis S  CTTCTGATGT TGCTGAATGA ACACGTGAGC GCACTGTGCA TCATTGCGTG CAGGGCCCGT CTCCTACCGG  140
An. fluviatilis T  .......... .......W.. .......... .......... .......... .......... ..........  140
An. fluviatilis U  .......... .......A.. .......... .......... .......... .......... ..........  140
An. minimus C      ..A....... .......... .......... .......... .......... .......... ..........  140

An. fluviatilis S  GAACCTTGGG CGCTGAAA-A GGTAAGGCAG TACAGTTCCA CTGTACAATT TGGGGG-TGC AGCGTCAAGT  208
An. fluviatilis T  .......... ........C. .......... .G....GTT. .......... ....K.G... ..........  210
An. fluviatilis U  .......... ........A. .......... ......GTT. .......... .....G.... ..........  210
An. minimus C      .......... ........A. .......... ......GT.. .......... ......-... ..........  209

An. fluviatilis S  CGCACGGGTC GAACTTCGGC TATGGACGAC CTGAGATACC CGGCAGCCTA CTAACACCAG GCTTGTCGAC  278
An. fluviatilis T  .......... .......... .......... .......... .......... .......... .........T  280
An. fluviatilis U  .......... .......... .......... .......... .......... .......... .........T  280
An. minimus C      .......... .......... .......... .......... .......... .......... ..........  279

An. fluviatilis S  CAGGTTCCAG GGGTTACGAA TCATCCGGCC GAGTCGTGTA ACGCGTG-CG ACCCATACGG TGCACCCATG  347
An. fluviatilis T  A......... .......... .......... .......... .......-.. .......... ..........  349
An. fluviatilis U  A......... .......... .......... .......... .......-.. .......... ..........  349
An. minimus C      .......... ........C. .......... .......... .......A.. .......... .......G.A  349

An. fluviatilis S  TTTAGATGGC AACCTACCTT CATAA  372
An. fluviatilis T  ....AT.... .......... T.C..  374
An. fluviatilis U  W...AT.... .......... T.C..  374
An. minimus C      ..G..TG... .....T.... TC..C  374
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Fig. 1.3: (a) Aligned nucleotide sequence of cytochrome oxidase II; and (b) internal transcribed spacer 2 from members of the Fluviatilis Complex and *An. minimus* C. The dot (.) represents similarity of nucleotide with *An. fluviatilis* S and dash (-) missing nucleotide. The *An. minimus* C nucleotides that differ from *An. fluviatilis* S are highlighted

regions. The COII region was PCR-amplified using primers Leu (5′-TCT AAT ATG GCA GAT TAG TGC A-3′) and Lys (5′-ACT TGC TTT CAG TCA TCT AAT G-3′). The ITS2 region of rDNA was amplified using primers ITSA (5′-TGT GAA CTG CAG GAC ACA T-3′) and ITSB (5′-TAT GCT TAA ATT CAG GGG GT-3′). For each PCR assay, we used 50 µl PCR reaction mixture which contained 0.5 µM of each primer, 200 µM of each dNTP, 1.5 mM of $MgCl_2$ and 1.25 unit of Taq Polymerase. The conditions for both PCRs were—an initial denaturation at 95°C for 5 min followed by 35 cycles at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min followed by a final extension at 72°C for 7 min. The PCR products were purified using Quiaquick PCR Purification Kit to remove unincorporated primers and dNTPs prior to sequencing. The sequencing was done on both strands of amplified DNA using BigDye v3.1 Terminator Cycle Sequencing Ready Kit following manufacturer's protocol. The primers used for sequencing were same as those used in the original PCR amplification. The other sequences of members of the Minimus and Fluviatilis Complexes for COII, ITS2 and 28S-D3 rDNA were downloaded from GenBank.

DNA sequences were aligned using the ClustalW method implemented in software Molecular Evolutionary Genetics Analysis version 3.1 (MEGA 3.1). The ITS2 and COII sequences were used for paired distance analysis independently for each locus as well as together along with 28S rDNA sequences. Genetic distances were estimated by the Kimura 2–parameter model using software MEGA 3.1. Sites containing alignment gaps were not used in the distance analyses and were treated as missing information. The Neighbour-Joining (NJ) and Maximum Parsimony (MP) trees were constructed using Kimura 2-parameter model including transitions and transversions. The strength of internal nodes was estimated by 1000 bootstrap replicates. The sequences of *An. aconitus* and *An. varuna* were taken as outgroups during construction of the trees.

The DNA sequence data of *An. fluviatilis* S for COII and ITS2 are shown in Fig. 1.3 along with the

Table 1.1. Pair-wise distance (below diagonal) and number of nucleotide difference (above diagonal) among members of the Fluviatilis Complex and *An. minimus* C

	An. fluviatilis S	*An. fluviatilis* T	*An. fluviatilis* U	*An. minimus* C
(a) COII				
An. fluviatilis S	–	2	2	22
An. fluviatilis T	0.003 [0.002]	–	4	24
An. fluviatilis U	0.003 [0.002]	0.006 [0.003]	–	22
An. minimus C	0.033 [0.007]	0.036 [0.008]	0.033 [0.007]	–
(b) ITS2				
An. fluviatilis S	–	10	9	13
An. fluviatilis T	0.028 [0.009]	–	1	15
An. fluviatilis U	0.025 [0.008]	0.003 [0.003]	–	14
An. minimus C	0.036 [0.010]	0.042 [0.011]	0.039 [0.011]	–
(c) Combined (COII, ITS2 and 28S-D3 rDNA)				
An. fluviatilis S	–	15	14	35
An. fluviatilis T	0.011 [0.003]	–	6	42
An. fluviatilis U	0.010 [0.003]	0.004 [0.002]	–	39
An. minimus C	0.026 [0.004]	0.031 [0.005]	0.029 [0.005]	–

Figures in bracket indicate standard error.

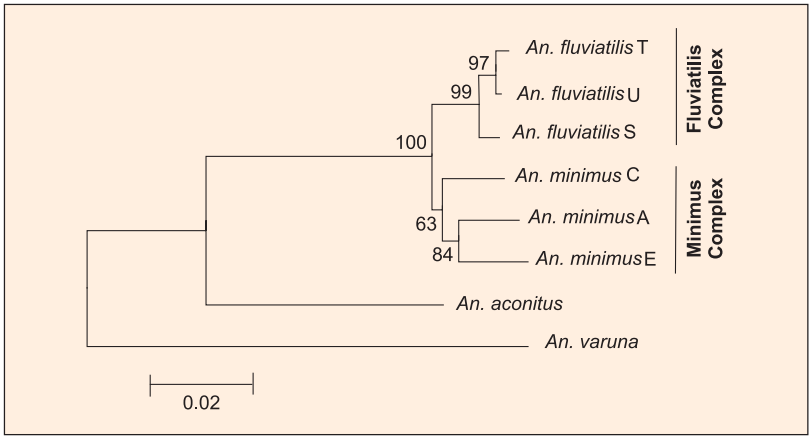


Fig. 1.4: Neighbour-Joining tree inferred from combined sequences data of the three loci (COII, ITS2 and 28S-D3) from members of the Fluviatilis and Minimus Complexes. Numbers above the branches are bootstrap values

sequences of *An. minimus* C and other members of the Fluviatilis Complex. There were two haplotypes of COII in *An. fluviatilis* S (GenBank accession numbers: DQ383278 and DQ383279) differing by one base pair substitution, however, there was no difference in deduced amino-acid sequence. The data shown in Fig.1.3 belong to majority haplotype (representing six out of seven samples sequenced). The ITS2 sequences of all the six specimens of *An. fluviatilis* S sequenced were identical (GenBank accession number DQ345964).

The pair-wise distances among *An. minimus* C and members of the Fluviatilis Complex, as computed separately for COII and ITS2 locus and combined for 28S-D3, CO II and ITS2 loci are shown in Table 1.1. Comparison of sequences revealed that *An. fluviatilis* S differs from *An. minimus* C by 22 base substitutions in COII and by 13 substitutions and two indels in ITS2 region. However, this species differs from other members of Fluviatilis Complex (species T and U) by only 2 and 9–10 base pair substitutions, respectively, for COII and ITS2 regions. In combined analysis, a total of 1388 aligned base pairs data were used including 328 base pair 28S-D3 sequence data where *An. fluviatilis* S and *An. minimus* C have identical sequences. In combined data analysis, *An. fluviatilis* S differs from *An. minimus* C by 35 base pair substitutions (2.6%), while it differs from species T and U by 15 (1.1%) and 14 (1%) base pairs only indicating that *An. fluviatilis* S is closely related to members of the Fluviatilis Complex as compared to *An. minimus* C.

The NJ tree for members of Fluviatilis and Minimus Complexes derived from combined set of data (COII, ITS2 and 28S-D3) shows that the Fluviatilis Complex (along with species S) forms a monophyletic taxon which is distinctly separated by Minimus Complex with nodal support of 100% bootstrap value (Fig. 1.4). Similar trend was obtained when we used MP method for phylogenetic reconstruction (data not shown). These results clearly establish the specific status of *An. fluviatilis* S as one of the member of Fluviatilis Complex and revokes it's synonymy with *An. minimus* C.

1.2 Insecticide-resistance

1.2.1 Insecticide-resistance studies in Surat district (Gujarat)

Field studies were carried out in the villages in Ukalda PHC area, District Surat in November 2005 to assess the status of insecticide-resistance in *An. culicifacies*. Earlier studies indicated development of

resistance to deltamethrin in 2002 in addition to the existing resistance to DDT and malathion. In these villages insecticide indoor residual spray is not being carried out for the past 5–6 years. This study was conducted to assess the present status of resistance to the insecticides used in public health. Studies included determination of base line resistance to different insecticides, synergistic studies, sibling species composition and differential insecticide-susceptibility in sibling species.

Base line resistance

This was determined using the standard WHO method and kit by exposing the mosquitoes to the designated WHO diagnostic doses of different insecticides. In susceptibility tests *An. culicifacies* was found 40% susceptible to DDT (n = 93); malathion—95% (n = 142); fenitrothion—97% (n = 146); propoxur—95% (n = 133); bendiocarb—100% (n = 107); deltamethrin—98% (n = 97); lambda-cyhalothrin—97% (n = 116; cyfluthrin—96.8% (n = 116) and to permethrin it was 99.2% (n = 140). Thus, the species was found resistant to DDT and malathion and was susceptible to other organophosphate, carbamate and pyrethroid insecticides.

Synergistic studies

Synergistic bioassay studies with tri phenyl phosphate (TPP), a specific inhibitor of carboxyl esterase indicated complete synergism with TPP (10%). This indicated the involvement of carboxyl esterase for conferring malathion resistance in this species. The recorded mortality for malathion alone was 66%, while exposure to TPP (5%) followed by exposure to malathion (5%) impregnated papers the mortality increased to 95%. Similarly, with pre-exposure to TPP (10%) followed by malathion it was 100%.

Susceptibility in sibling species

Female *An. culicifacies* mosquitoes and the dead and alive mosquitoes in the insecticide exposures in WHO susceptibility tests were stored in isopropanol and were later identified to sibling species using PCR assays. The species composition of *An. culicifacies* indicated major sympatricity of species B and E. The proportion of species E was 59.5%, species B was 39.3% and species C was only 1.2%. Prevalence of species E from this area is being reported for the first time. Identification of dead and alive mosquitoes in the insecticide bioassays to sibling species indicated differential susceptibilities to DDT and malathion. To DDT, species B was 31.5% susceptible, while to malathion it was 64% and species E was 56.2 and 75% susceptible to DDT and malathion respectively (Table 1.2). Thus, species B was relatively less susceptible to DDT and malathion. Susceptibility in species C could not be determined due to its very low prevalence.

Table 1.2. Susceptibility status of *An. culicifacies* species B and E to different insecticides in District Surat

Species	Insecticide, time	% Mortality (n)
An. culicifacies s.l.	DDT (4%), 1 h	40.0 (93)
Species B	DDT (4%), 1 h	31.5 (57)
Species E	DDT (4%), 1 h	56.2 (32)
An. culicifacies s.l.	Malathion (5%), 1 h	66.0 (142)
Species B	Malathion (5%), 1 h	64.0 (51)
Species E	Malathion (5%), 1 h	75.0 (12)

n: Number exposed.

1.3 Vector control

1.3.1 Evaluation of PermaNet® 2.0 against malaria vectors in India

PermaNet, a long-lasting insecticide treated net (LLIN) treated with deltamethrin @55 mg/m^2 was evaluated for bioefficacy against malaria vectors and other mosquitoes in malaria endemic areas of Uttar Pradesh. For it, the study was conducted in Nawada, Harampur and Dugrawali villages from April to November 2005. Percent corrected mortality of *An. culicifacies* and *An. stephensi* in three minute contact bioassays performed on unwashed and washed PermaNet 2.0 are depicted in Fig. 1.5. The results showed high efficacy of PermaNets against both the

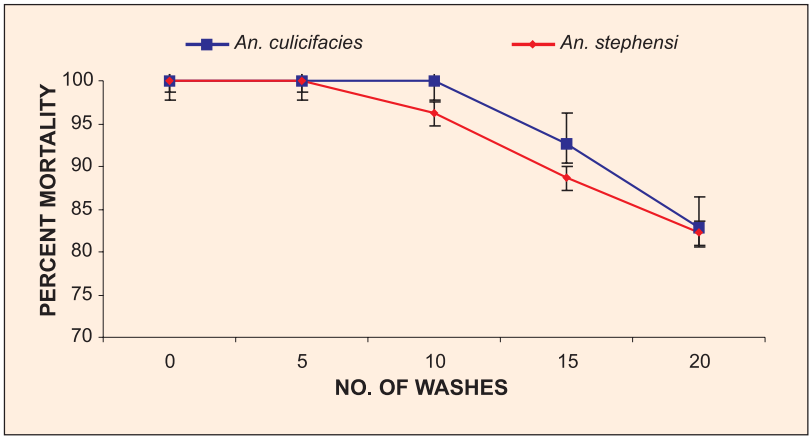


Fig. 1.5: Percent mortality (24 h holding) of mosquitoes exposed to unwashed and washed PermaNet 2.0 in three min contact bioassays

species as evidenced by the fact that even after 20 washes the mortality in both the species remained >80%. Further, there was no significant difference between the mortalities of the two species ($p > 0.05$). In ring net bioassay, the base line median knockdown time (MKDT) against *An. culicifacies* was 390 sec. However, after progressive washings the MKDT was also increased. The MKDT of *An. culicifacies* was less than that of *An. stephensi* and the difference was statistically significant ($p < 0.05$) (Table 1.3).

Use of PermaNets also reduced the man hour densities (MHD) of different mosquito species. The results revealed that the MHD of *An. culicifacies* and *An. stephensi* in the structures selected for PermaNet, plain net and without net during pre distribution period was 27 and 53, 22 and 32 and, 20 and 22 respectively. After the distribution of nets the densities reduced gradually in PermaNet used structures. Though there was reduction in density in structures where the plain nets were used, the impact was more pronounced in PermaNet used structures. PermaNet showed high excito repellent activity (82–97%) against *An. culicifacies*, 77–97% against *An. stephensi*. However, the repellent action was below 30% against all the mosquito species. PermaNet has high killing activity as evidenced by the fact that all the landed mosquitoes on the net died after 24 h of observation. About 20 to 40% mortality was observed in mosquitoes that entered the room and were accidentally exposed to the net and rested on the walls. The results clearly demonstrated that PermaNet showed high efficacy in producing mortality in the mosquitoes, which were exposed to the net.

Table 1.3. Medium knockdown time (in sec) of *An. culicifacies* and *An. stephensi* exposed to unwashed and washed PermaNets

Type of net	*An. culicifacies*	*An. stephensi*
Unwashed PermaNet	390 ± 13	480 ± 22
5 times washed	397 ± 18	548 ± 32
10 times washed	415 ± 16	602 ± 26
15 times washed	448 ± 21	727 ± 17
20 times washed	472 ± 22	968 ± 28

Each value is mean ± S.D. of four replicates.

Safety of PermaNets usage was studied by interviewing the users of the nets through structured questionnaires. Results revealed that eye and skin irritation were the main complaints reported by the inhabitants. Further the inhabitants also mentioned that these symptoms were not persisted after washing the affected areas with clean water. This may be due to high dose of deltamethrin on the PermaNet. Symptoms like headache, vomiting, nausea, etc. were not reported.

1.3.2 Phase III evaluation of Olyset nets for malaria control

The operational feasibility and efficacy of Olyset net (LLIN) was studied against *An. culicifacies*, a principal malaria vector that transmits 65% of total malaria cases in rural areas of India. Three villages in District Gautam Budh Nagar (Uttar Pradesh), India were selected for the trial and Olyset nets were distributed in one village, in other village plain nets were distributed and the third village was kept as control where nets were not used. Entomological and epidemiological data were collected using standard methods. Altogether 1203 Olyset nets were distributed among 701 males and 502 females aged above 10 years in Khandera village and 1289 untreated nets among 719 males and 570 females aged above 10 years in Beel Akbarpur village.

Usage pattern of nets by the inhabitants in the study villages is shown in Fig. 1.6. It was found that the inhabitants were regularly using nets as there was always > 98% usage during transmission season (August to November), and also in the months of March and April when the *Culex* mosquito densities are generally high. In this area in winter season (January and February) the density of *Anopheles* mosquitoes is generally very low due to extremely low temperatures and people generally do not use nets. About 2.5% Olyset nets were lost as against 1.04% untreated nets.

Entomological results revealed that use of Olyset nets has led to significant reduction in mosquito densities in general and particularly *An. culicifacies* ($p < 0.05$). There was a significant difference in the densities of *An. culicifacies* after distribution of nets when compared to those prior to distribution ($p < 0.05$) in both Olyset and untreated net distributed villages. Whereas in the control village the difference between pre-densities and post-densities was statistically insignificant ($p > 0.05$). In case of total mosquitoes, significant difference was observed in pre-densities and post-densities in Olyset net village only. When the post-densities were compared in between the villages, no statistically significant difference was observed in all combinations except between control and untreated nets.

The entry of *An. culicifacies* substantially reduced in Olyset net distributed village in comparison to untreated net village ($p < 0.05$). This was also reflected when the landing rate of *An. culicifacies* on both types of nets was compared in different villages. The mean landing rate on the Olyset net was nil whereas on the untreated net it varied from 8 ± 1.2 to 16.5 ± 2.5 mosquitoes/bait/night. Similarly, the mean landing rate of total mosquitoes was nil on the Olyset net as against 62 ± 9.8 to 125 ± 23.9 on plain net suggesting thereby that Olyset net has either airborne action or strong repellent action against mosquitoes in general and against *An. culicifacies* in particular.

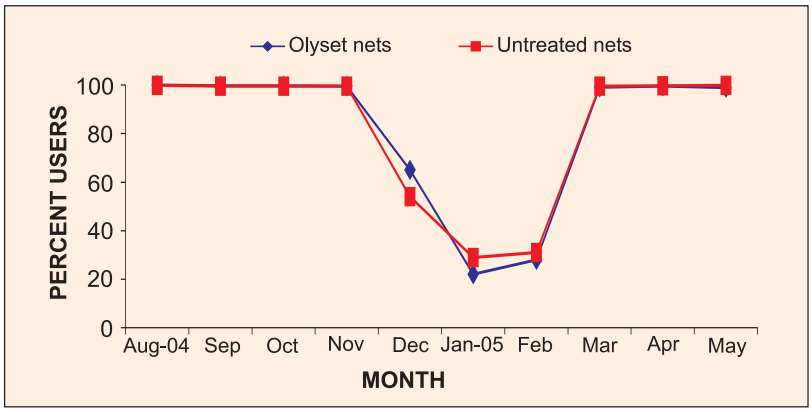


Fig. 1.6: Usage pattern of nets in the study villages

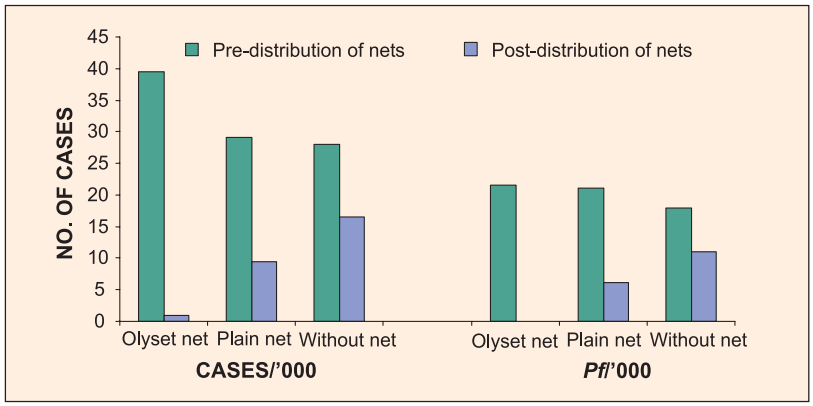


Fig. 1.7: Impact of Olyset nets on malaria incidence

There was a considerable reduction in the parity and sporozoite rates of *An. culicifacies* in the Olyset net distributed village in comparison to plain net distributed and control villages. There was statistically significant difference when the parity rates of *An. culicifacies* in both Olyset net and untreated net villages were compared. This clearly indicates that longevity of vector mosquitoes reduced drastically in the Olyset net distributed village. Further nil sporozoite rates in vector species indicate interrupted transmission in the villages where Olyset nets were used, whereas in the control village malaria transmission continued. These results clearly showed that use of Olyset nets protected the community and reduced the longevity of vectors.

Epidemiological results revealed that after introduction of Olyset nets in Khandera village in the month of August 2004, malaria incidence started declining and impact on SPR and SFR was quite high. There was no significant difference in SPR and SFR in all the villages prior to distribution of nets ($p > 0.05$). However, the SPR reduced drastically in the Olyset net distributed village after introduction of nets ($p < 0.1$ & > 0.05). Similarly significant difference at 0.1 level was observed only in between Olyset net and control village. At 0.05 level of significance the comparisons were insignificant.

Results of mass blood surveys carried out in these villages clearly demonstrated that malaria incidence was completely reduced when compared to that in pre-distribution mass blood survey in the Olyset net distributed village, whereas in the plain net and without net villages malaria cases were reported in both surveys after the distribution of nets (Fig. 1.7). These results further substantiated that Olyset nets are quite effective in controlling malaria. No adverse effect such as itching, nausea, vomiting, etc. was reported by the inhabitants using Olyset nets.

1.3.3 Evaluation of Dimilin (Diflubenzuron) GR-2 (2% granules) and TB-2 (2% tablets) for control of mosquito larvae

Two formulations of Dimlin (N-4 chlorophenyl amino carbonyl-2-6 difluro-benzamide)—GR-2 (2% granules) & TB-2 (2% tablets) supplied by M/s. Crompton Uniroyal Chemicals Asia Pacific Pvt. Ltd., Mumbai were tested for their bioefficacies against immatures of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* in their respective breeding habitats.

Field evaluation was carried out in specific breeding places of target species in and around Delhi. Habitats with predominant breeding of a particular species were selected for these trials. Since it was not possible to observe mortality of larvae, pupae and adults in natural habitats, therefore, these water bodies were initially treated with required con-

Application of Dimilin granule formulation in pits

Application of Dimilin tablet formulation in cooler

centration on volume basis for tablet formulation in contained water habitats such as tanks, and on area basis for granule formulation in other habitats. Water samples from treated habitats were brought to the laboratory on the same day and Day 3 and later at an interval of one week. Laboratory colonised late III instar larvae were introduced in subsequent weeks till the complete pupation or adult emergence under controlled conditions. Also late instar larvae collected from the treated habitats were observed till the complete pupation or adult emergence under controlled conditions. Percent inhibition was calculated on the basis of untreated control run concurrently.

Results of field evaluation revealed that application of Dimilin TB-2% @ ½ tablet and 1 tablet per 40 litre (L) water (equivalent to 0.5 & 1 ppm) produced complete inhibition of the development of pupae and emergence of adult mosquitoes in tanks with clear water against *An. stephensi, Ae. aegypti* and also *Cx. quinquefasciatus* up to 4 weeks of observation. When used @ 1 tablet in 400 L of water (equivalent to 1 ppm) 100% inhibition of adult emergence was observed against *An. stephensi* up to 7 days and against *Ae. aegypti* and *Cx. quinquefasciatus* up to 14 days and 98–99% inhibition was recorded up to 4 weeks. The efficacy of Dimilin GR-2 formulation in cement tanks when treated @ 1.5 kg/ha also showed 100% inhibition of adult emergence in case of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* up to 4 weeks (Figs. 1.8–1.10). However, at lower dose—1 kg/ha, 100% inhibition

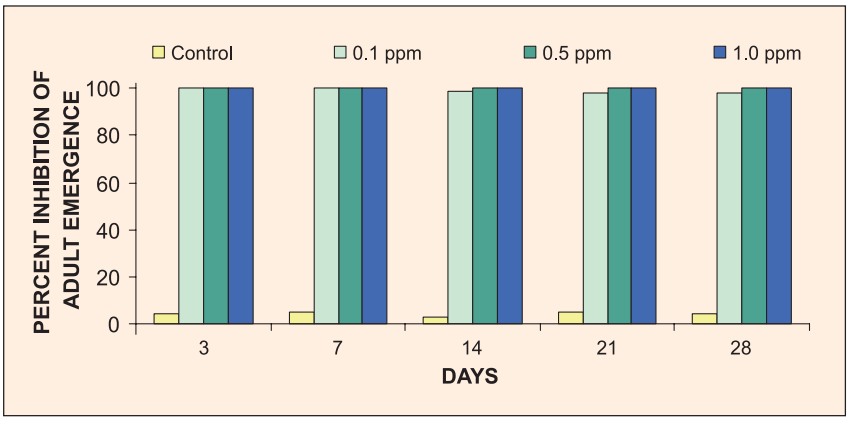


Fig. 1.8: Efficacy of Dimilin TB-2 against *An. stephensi* in cement tanks

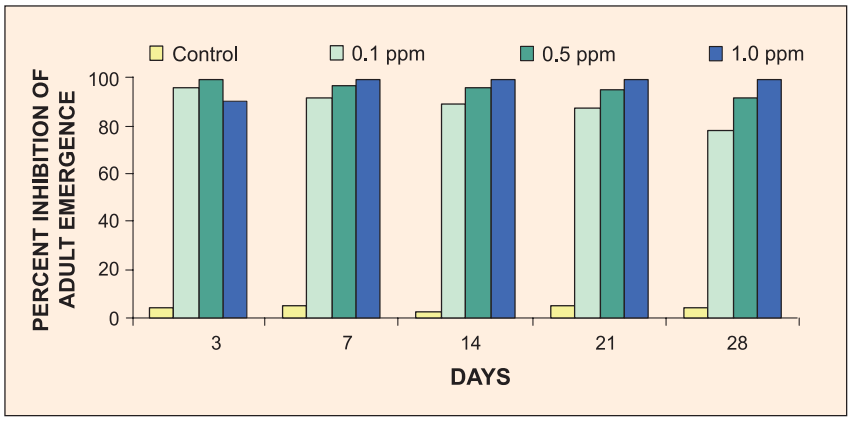


Fig. 1.9: Efficacy of Dimilin GR-2 against *An. stephensi* in cement tanks

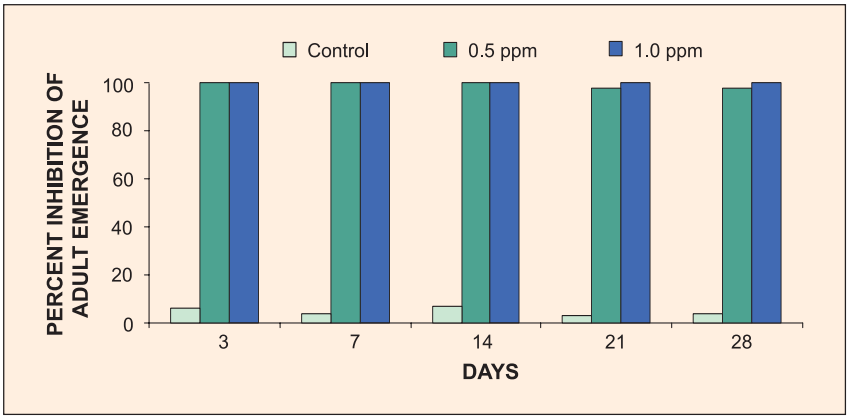


Fig. 1.10: Efficacy of Dimilin TB-2 against *Ae. aegypti* in desert coolers

was observed against *Cx. quinquefasciatus* up to 3 days only, whereas against *An. stephensi* and *Ae. aegypti* only 97 and 96% inhibition was observed on Day 3 respectively. It may be pointed out that only higher dose of granule formulation—1.5 kg/ha was effective in clean water in cement tanks for complete inhibition of adult emergence up to four weeks.

Field evaluation of Dimilin 2% tablet against immatures of *Anopheles* spp and *Cx. quinquefasciatus* in moderately polluted small pools also showed 100% inhibition of adult emergence up to 4 weeks of observation @ 0.5 & 1 Tab/40 L water. However, @ 0.1 Tablet/40 L (1 Tab/400 L) the percent inhibition of adult emergence ranged between 92 and 98 against both the species during the four weeks of observations.

Dimilin 2% Granules against larvae of *Anopheles* spp in pools @ 1.5 kg/ha produced 100% inhibition of adult emergence of *Anopheles* larvae up to 4 weeks of observation but at lower doses—1 & 1.25 kg/ha, the percent inhibition was 86–92 after 3 days and this effect gradually diluted as evidenced by the fact that after 4 weeks 80–84% emergence was recorded. Dimilin GR-2 @ 1.5 kg/ha against *Cx. quinquefasciatus* larvae in pools produced 100% inhibition of adult emergence up to 2 weeks. However, at lower doses only 86–89% inhibition was observed on Day 3 itself which declined to 73–81% after 4 weeks (Fig. 1.11).

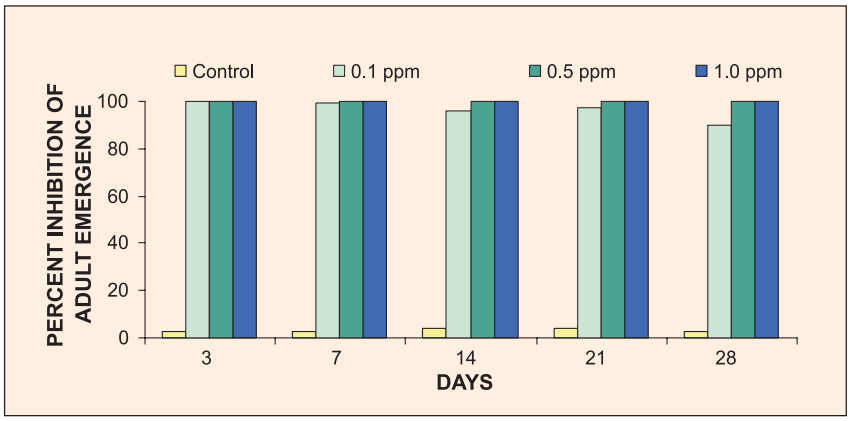


Fig. 1.11: Efficacy of Dimilin GR-2 against *Cx. quinquefasciatus* in cement tanks

1.3.4 Larvicidal activity of extracts of different parts of a tissue cultured plant (code VA-1 of family Astereaceae)

Studies were carried out in collaboration with University of Delhi to assess the larvicidal activity of the hexane extract of root, flower, leaf and stem of the coded VA-1 plant (Family: *Astereaceae*). The efficacy was tested against two mosquito species, *An. stephensi* and *Cx. quinquefasciatus*. The calculated LC_{50} and efficacy of different parts against *An. stephensi* was in the order—root (2.71 ppm) > flower (7.38 ppm) > leaf (61 ppm) > stem (86.92 ppm). For *Cx. quinquefasciatus* the values were: root (1.19 ppm)> flower (8.52 ppm) > leaf (14.54 ppm) > stem (26.33 ppm). The order of efficacy for different parts in the two species was same but the overall efficacy of the formulations of different parts was more against *Cx. quinquefasciatus* (Table 1.4).

1.3.5 Study to assess the repellency of a natural oil formulation (DRP-I) on disease mosquito vectors

Cage studies were carried out to assess the repellency of a natural oil formulation (DRP-1) in five vector species namely *An. culicifacies* species A, and C, *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. Relative repellency studies were carried out against three concentrations of the natural oil formulation (DRP-1)—2.5, 5 and 10% and a known chemical repellent (DEET) at 2.5%. Observations of the relative repellencies were made at 0 h and subsequently at 1, 2, 4 and 6 h. This was done by recording the number of mosquitoes landing on the impregnated oil and DEET in 10% glucose soaked cotton ball placed in Styrofoam glasses. Glucose alone was the control in the study. A total of 100, 5–6 day old sugar-fed female mosquitoes were intro-

Table 1.4. Larvicidal activity (ppm) of different concentrations of the crude hexane extract of different parts of the tissue cultured plant VA-1 of family Astereaceae against *An. stephensi* and *Cx. quinquefasciatus*

Part	*An. stephensi*			*Cx. quinquefasciatus*		
	Conc. (Range)	LC_{50}	LC_{90}	Conc. (Range)	LC_{50}	LC_{90}
Root	7.5 (0.29–15)	2.71	4.26	3.75 (0.29–7.5)	1.19	2.06
Flower	33.3 (0.26–66.4)	7.38	13.52	16.66	8.52	14.27
Leaf	105 (15–210)	61.0	95.0	71.6 (1.1–142)	14.54	27.7
Stem	220 (50–440)	87.0	110	110 (0.86–220)	26.33	47.83

Mosquito cage showing Styrofoam glasses with control and repellent soaked cotton

Recording of landing rates of mosquitoes on control and repellent soaked cotton

duced into 2 sq ft cloth cage and the Styrofoam glasses with the oil impregnated cotton, DEET impregnated and glucose alone were placed in different corners of the cage. Number of mosquitoes landing on cotton impregnated with different concentrations of DRP-1 and glucose were simultaneously counted. DRP-1 was found effective in repelling the species of mosquitoes in the observations made at different hours of observations (Table 1.5). The percent repellency observed at different observation periods ranged from 80–100% against different concentrations of repellents and different species. *Ae. aegypti* and *Cx. quinquefasciatus* have shown >95% repellency to DRP-1 at all the concentrations—2.5, 5 and 10%. *An. culicifacies* species A, *An. culicifacies* species C and *An. stephensi* have shown 93–100% repellency against both 5 and 10% concentrations of DRP-1. From the observed data on the repellency against the five important disease vectors it can be concluded that a dose of 5% could be used for achieving the desired level of protection against bites of these mosquitoes. However, these results pertain to the effectiveness in cage experiments using only sugar solution and need further confirmation by testing the same on human subjects to testify the observed repellency against the natural host.

Table 1.5. Repellency rates against different concentrations of repellents in different species

Species	DEET (2.5%)	DRP (2.5%)	DRP (5%)	DRP (10%)
An. culicifacies species A	100	84–100 (6 h)	93–100 (4, 6 h)	96–100 (0, 2, 4 h)
An. culicifacies species C	90–100 (1, 2, 4 h)	83–100 (1, 2, 4 h)	100	100
An. stephensi	85–100 (6 h)	85–100 (6 h)	92–100 (0, 2, 4 h)	97–100 (0,1,4,6 h)
Cx. quinquefasciatus	80–90 (–)	95–100 (0,1 h)	100	100
Ae. aegypti	93–97 (–)	99–100 (0,12 h)	100	100

Figures in parentheses indicate time period.

1.3.6 Antifungal activity of neem oil against entomopathogenic fungi and its possible application in integrated vector control programme

The growth inhibitory action of neem oil against the entomopathogenic fungi *Lagenidium giganteum* and *Metarhizium anisopliae* was studied. The toxicity of the neem oil to mycelia growth of *L. giganteum* and *M. anisopliae* is shown in Fig. 1.12. The IC_{50} of neem oil was compared among the three media namely peptone-yeast extract-glucose (PYG), Emerson's YpSs and Sunflower seed (SFS). It was observed that the growth of *L. giganteum* was 3.9- and 2.4-fold more than the *M. anisopliae* in PYG and Em YpSs media respectively. When grown in SFS media, neem oil did not inhibit the growth of *L. giganteum*. Minimum inhibitory concentration (MIC) of the neem oil against *L. giganteum* and *M. anisopliae* in the three broths are shown in Table 1.6. The MIC of *L. giganteum* was 3- and 2-fold higher than the MIC of *M. anisopliae* in PYG and Em YpSs respectively. In SFS broth, neem oil did not inhibit the growth of *L. giganteum*. However, it was more effective in inhibiting the growth of both the species in Em YpSs than in PYG broth. The minimum fungicidal concentration (MFC) was achieved at much higher concentration than the MIC (Table 1.6). *L. giganteum* growth in SFS was unaffected in the presence of neem oil, whereas the growth of *M. anisopliae* was restricted at various concentrations of neem oil. These studies have indicated that the neem oil inhibits its mycelial growth in PYG and Em YpSs media, while in SFS medium no inhibition of *L. giganteum*

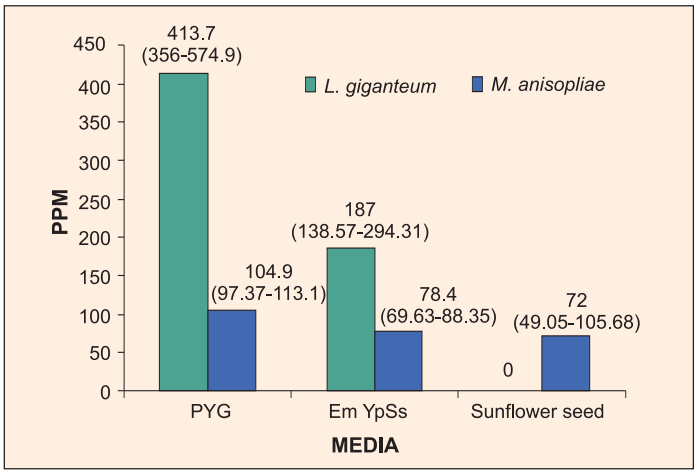


Fig. 1.12: Growth inhibition concentration (IC_{50}) of neem oil in ppm against *L. giganteum* and *M. anisopliae* in three different media (95% fiducial limit in parentheses)

Table 1.6. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of neem oil (µl/ml) against *L. giganteum* and *M. anisopliae* after 24 h of incubation

Strain	MIC			MFC		
	PYG	Em YpSs	SFS	PYG	Em YpSs	SFS
L. giganteum	30	20	–	60	60	–
M. anisopliae	10	10	–	40	60	40

was observed. This study provides a lead to a successful Integrated Vector Control Management with neem oil as a candidate vis-à-vis the fungal strains.

1.3.7 Isolation of *Bacillus* strains from soil and their efficacy against *An. culicifacies*

Soil samples were collected from locations in the vicinity of mosquito breeding habitats of Delhi and Himachal Pradesh in asceptical conditions. Five strains of *Bacillus* were isolated from the Delhi soil and three from the Himachal Pradesh. Out of five strains of Delhi, two were found to be entomopathogenic and coded as MB-1 and HB-1. One strain of Himachal Pradesh was found to be effective against mosquitoes and coded as HP-1. The efficacy of the three strains against *An. culicifacies* species A was tested after growing them for 48 hours in LB + NYSM media. The LC_{50} of the three strains are shown in Table 1.7. Among the three strains, MB-1 was found to be more effective than the other two strains. These coded strains have been sent to MTCC (IMTECH), Chandigarh for identification.

Table 1.7. LC_{50} of coded *Bacillus* strains (in µl/ml) against II instar larvae of *An. culicifacies* species *A* after 24 h of inoculation

Name of coded strain	LC_{50}	LC_{90}
MB-1	28.87 (20.5–34.92)	45.05 (36.92–78.59)
HB-1	62.33 (56.69–67.82)	84.84 (77.03–98.36)
HP-1	56.04 (47.76–65.67)	286.42 (209.65–308.84)

1.3.8 Studies on larvicidal properties of aqueous leaf extract of *Xanthium stramorium* (Family: Aizoaceae)

Larvicidal activity of crude aqueous extract of the leaf of a medicinally important plant *Xanthium stramorium* was tested against III/IV instar larvae of *An. culicifacies* species A and C, *An. stephensi* and *Cx. quinquefasciatus* following standard WHO method for a range of concentrations. The calculated LC_{50} (lethal concentration for killing 50% of treated larvae) for different species were respectively: *An. culicifacies* species A—0.19%, *An. culicifacies* species C—0.22%, *An. stephensi*—0.13%, *Cx. quinquefasciatus*—0.27% and *Ae. aegypti*—0.27% (Table 1.8).

1.3.9 Prospecting for botanical pesticides

The study was undertaken in collaboration with other institutes as a part of the DBT funded project "Prospecting for botanical pesticides". Plant extracts/ fractions/formulations obtained from other collaborating institutes under the project were screened at NIMR for their bioactivity against mosquitoes, particularly against malaria vector, *An. stephensi* using standard protocol.

Since beginning of this project, a total of 732 coded samples of different plant extracts/ fractions/ formulations were received at NIMR for bioassays against *An. stephensi*. Of these, 727 samples were

Table 1.8. Larvicidal activity of different concentrations of the crude aqueous extracts of the leaves of *Xanthium stramorium*

Species (instar)	LC_{50} (% Solution)	LC_{90} (% Solution)	χ^2 (df=6)
An. culicifacies A (III/IV)	0.19	1.3	123.88
An. culicifacies C (III/IV)	0.22	1.5	81.53
An. stephensi (III/IV)	0.13	0.8	33.18
Cx. quinquefasciatus (III/IV)	0.27	1.0	42.93
Ae. aegypti (III/IV)	0.27	1.8	86.95

Table 1.9. Samples and screening status of plant extracts/fractions

Laboratory/Institute	Received samples	Screened samples	Positive results		
			L	A	R
RRL (Trivendrum)	105	105	8	3	1
FRI (Dehradun)	121	121	32	16	4
IIT (Delhi)	187	182	75	2	–
RRL (Jammu)	170	170	28	6	–
EID Parry (Bangalore)	152	152	29	2	–
Total	732	727	172	29	5

L–larvicidal activity; A–Adulticidal activity; R–Repellent activity.

tested and their bioactivity is shown in Table 1.9. Of the 727 samples tested so far, 172 samples showed larvicidal activity (70–100% mortality) at 250 ppm, while 29 samples showed insecticidal activity against adult mosquitoes and 5 samples have shown repellent activity for more than one hour. Of the various samples tested, 4 plants were identified for further development of plant extract based formulations for testing against mosquitoes and other agricultural pests. About 83 formulated samples were received and tested against larvae of the two mosquito species—*An. stephensi* and *Ae. aegypti*. Most of the formulated samples, produced 100% mortality in the mosquito larvae at 250 ppm, but very low or no mortality was recorded at lower concentrations and, therefore, lethal concentrations could not be determined. None of these formulated samples showed activity against adult mosquitoes.

1.4 Vector immunity

1.4.1 Immune response in the haemolymph of *An. culicifacies* against the challenge of gram-positive bacteria *Micrococcus leutus*

We have earlier studied the immune response in the body tissue of species A, B and C of Culicifacies Complex upon challenge with gram-positive bacteria *M. leutus*. Further, another immune-responsive site—haemolymph which has a direct involvement in wound healing and combating invaders of the body cavity of mosquitoes was used for studying the immune response of species A, B and C upon *M. leutus* challenge. Four to six day old laboratory-colonised *An. culicifacies* mosquitoes were used along with appropriate control. Studies were carried out on three groups of mosquitoes, I group was kept as naïve, II group was given sterile injury and the III group was challenged with bacteria. Haemolymph was harvested after 1, 4 and 8 hours time period intervals post-injury. The tissues were processed for SDS-PAGE and were analysed for the changes in the polypeptide profile using Gene Tool™ software.

In species A, the sterile injury induced the synthesis of seven new polypeptides (220, 200, 198, 180, 176, 152, 120 kDa) and the expression of twelve naïve polypeptides ranging from 20 to 76 kDa was also enhanced (Fig. 1.13, Densitogram 1.1, Table 1.10). One 20.5 kDa polypeptide disappeared from the haemolymph samples after 8 h of sterile injury. *M. leutus* infection induced the synthesis of six new polypeptides (220, 198, 180, 176, 152 and 120 kDa) and activated ten existing polypeptides (76, 71, 56, 45, 37, 34, 32, 26.5, 24 and 22 kDa). In species B, injury induced two new polypeptides (18 and 11 kDa) and enhanced the expression of four existing polypeptides (37, 20.5, 19 and 6 kDa) from their basal level (Fig. 1.14, Table 1.11). In addition to the up regulation of these polypeptides, the injury also resulted in the down regulation of three naïve polypeptides (130, 122 and 10 kDa). In contrast, *M. leutus* infection stimulated the production of a novel

Table 1.10. Injury and bacteria inducible polypeptides of the haemolymph of species A of the Culicifacies Complex

A HI	220	200	198	180	176	152	120	76	71	56	45	43	37	34	32	26.5	24	22	20.5	20
N	–	–	–	–	–	–	–	+	+	+	+	++	+	+	+	+	+	+	+	+
1W	+	++	+	+	+	–	–	+	+	++	++	+	+	+	+	++	+	+	+	+
4W	+	++	++	++	++	+	+	++	++	++	++	++	++	++	++	++++	++	++	+	+
8W	+	–	++	++	++	+	+	++	++	++	++	+++	+	++	++	++++	++	++	–	++
1I	++	–	+	+	+	+	+	++	++	++	++	++	++	++	+	+++	++	++	+	+
4I	+	–	++	+++	+	+	+	++	++	++	++	++	++	++	++	++++	++	++	+	+
8I	–	–	++	+	++	–	–	+	+	++	+	++	+	+	+	+	+	+	+	+

Table 1.11. Injury and bacteria inducible polypeptides in the haemolymph of species B of the Culicifacies Complex

B HI	130	122	92	81	37	26.5	26	20.5	19	18	11	10	6
N	++	++	+	+	+	+	++	+	+	–	–	+	+
1W	+	+	+	+	+	+	++	+	+	–	+	-	+
4W	+	++	+	+	++	+	++	++	++	++	+	+	++
8W	++	++	+	+	++	+	++	+	+	++	+	+	++
1I	++	++	++	++	++	++	+	+	+	+	–	+	–
4I	+	+	+	+	++	+	–	+	+	+	–	+	–
8I	+	++	+	+	++	+	–	+	+	+	–	+	–

Table 1.12. Injury and bacteria inducible polypeptides in the haemolymph of species C of the Culicifacies Complex

C HI	198	180	152	81	56	45	43	32	26.5	24	11
N	+	+	+	++	+	++	++	–	+	+	+
1W	+	+	+	+	+	++	++	+	+	+	+
4W	+	+	+	+	+	+	++	–	+	++	+
8W	+	+	++	+	++	++	++	–	+	+++	++
1I	+	+	+	–	++	+	+	–	+	++	+
4I	+	+	+	+	+	-	+	–	+	++	++
8I	–	++	++	++	+	++	++	–	++	++++	++++

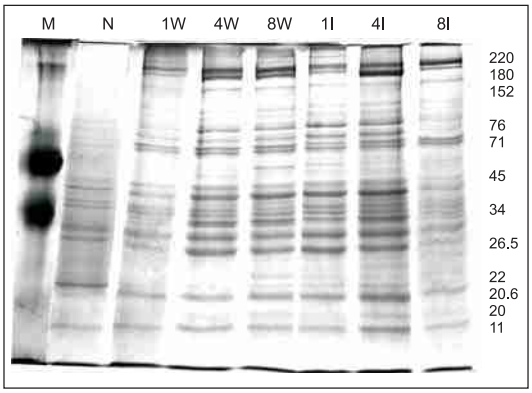


Fig. 1.13: Haemolymph of species A after injury with entomological needle contaminated with or without *M. leutus* (N : Naïve female; W: Sterile injury,1, 4 and 8 : Hours after treatment; I : Infected with *M. leutus* II)

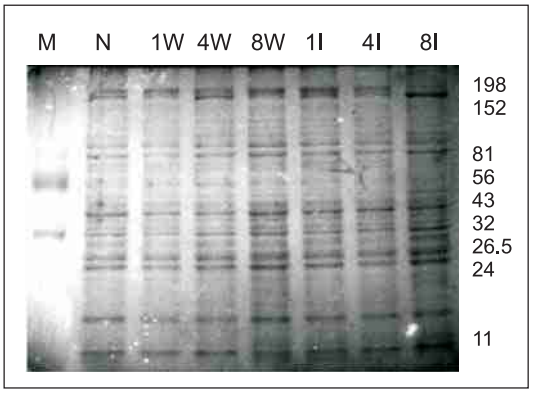


Fig. 1.15: Haemolymph of species C after injury with entomological needle contaminated with or without *M. leutus* (N : Naïve female; W: Sterile injury, 1, 4 and 8 : Hours after treatment; I : Infected with *M. leutus*)

18 kDa polypeptide and up regulated four polypeptides (92, 81, 37 and 26.5 kDa). Among all the up regulated polypeptides, a 26.5 kDa polypeptide was found to be up regulated many fold as compared to sterile injury in naïve mosquitoes. Three polypeptides; 130, 122 and 26 kDa were down regulated in response to bacterial infection. Sterile injury in species C resulted in the synthesis of 32 kDa, a new polypeptide and activated expression of four naïve polypeptides 152, 56, 24 and 11 kDa (Fig. 1.15, Table 1.12). Further, two polypeptides of molecular weight 81 and 45 kDa were found to be down regulated. The bacterial infection up regulated the expression of six naïve polypeptides 180, 152, 56, 26.5, 24 and 11 kDa. Further, four polypeptides of 198, 81, 45 and 43 k Da were down regulated.

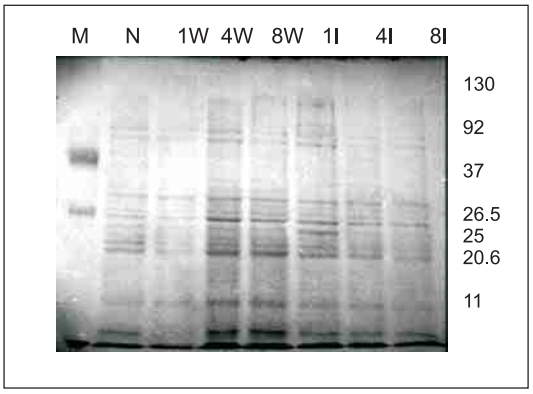
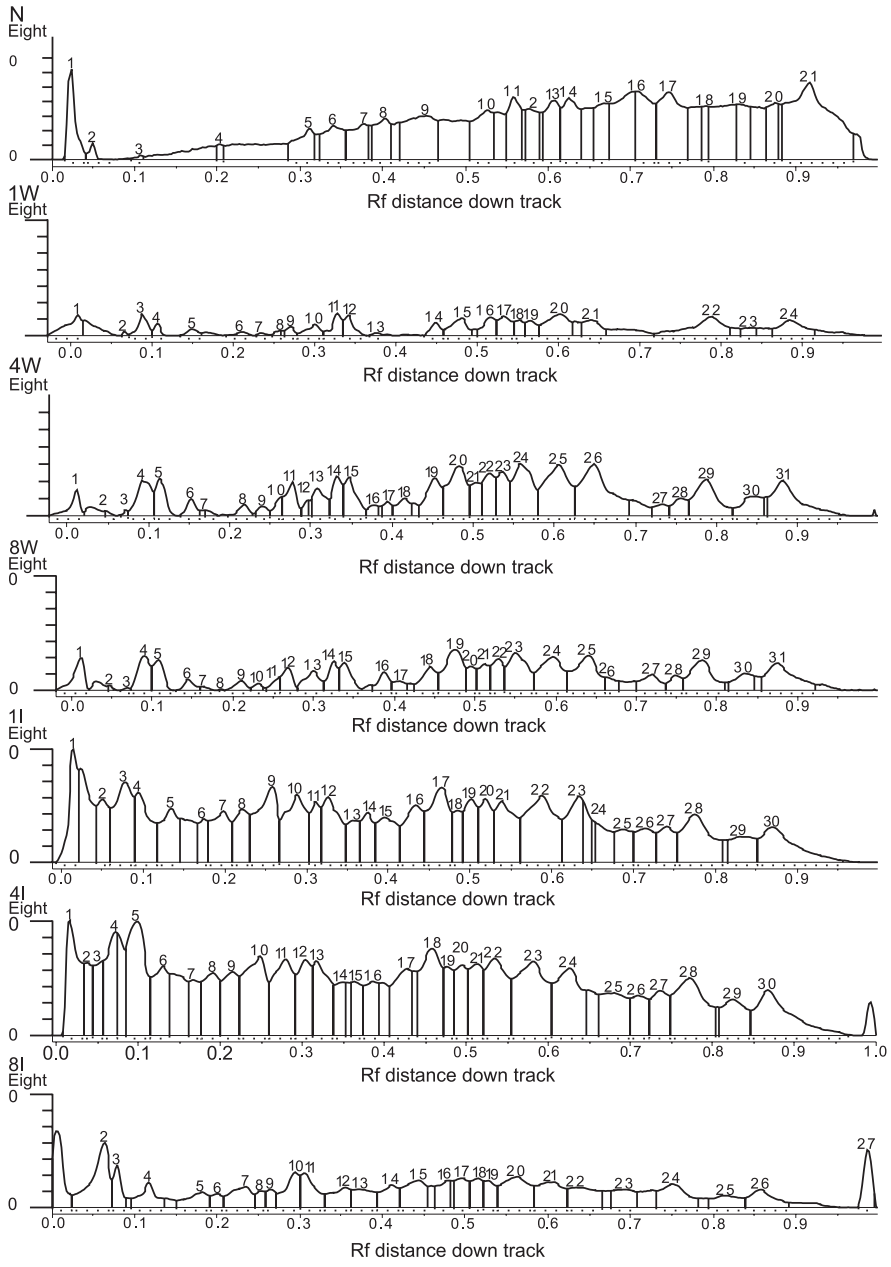


Fig. 1.14: Haemolymph of species B after injury with entomological needle contaminated with or without *M. leutus* (N : Naïve female; W: Sterile injury, 1, 4 and 8 : Hours after treatment; I : Infected with *M. leutus*)

The haemolymph of all these species responded differentially to sterile injury and against gram-positive bacterial challenge. Various injuries and *M. leutus*-responsive polypeptides detected in the haemolymph are candidates for future in-depth studies.

1.4.2 Quantitative analysis of enzyme phenoloxidase in *An. stephensi*

Phenoloxidase (PO) is one of the major enzymes of immune system in mosquitoes, whose expression can be seen phenotypically as melanisation—a phenomenon of deposition of melanin on the surface of invading foreign body. The expression of PO was studied in *An. stephensi* upon feeding on normal and *P. vinckei petteri* infected blood meal. For this purpose, 4–6 day old adult female mosquitoes from same batch were fed on healthy and *P. vinckei petteri* infected mice for one hour after nine hour starvation. Only full-fed mosquitoes were considered for the experiment. Body tissue (excluding midgut) was dissected in ice-cold sodium



Densitogram 1.1: Response of the haemolymph of *An. culicifacies* species A to injury and *M. leutus* infection (N : Naïve female; W: Sterile injury, 1, 4 and 8 : Hours after injury; I : *M. leutus* infection)

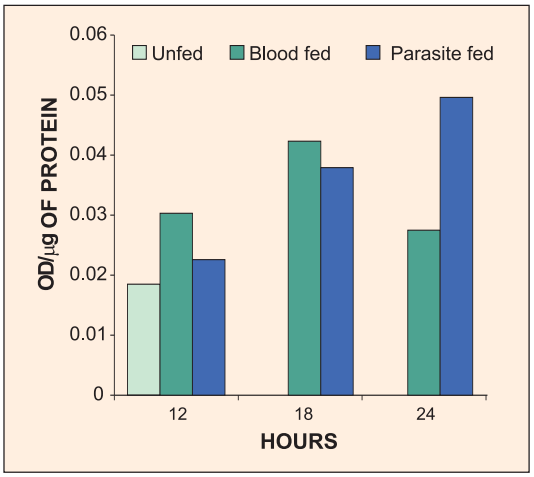


Fig. 1.16: Phenoloxidase activity (OD/µg of protein) in body tissue of *An. stephensi* upon normal and *P. vinckei petteri* infected blood meal

phosphate buffer (0.01 M, pH 7.2) at different time intervals (12, 18 and 24 h) post feeding and stored in liquid nitrogen. Tissues were homogenised in sodium phosphate buffer so as to obtain 25 µl supernatant. Part of the sample aliquot (5 µl) was used for protein estimation using Bradford method. PO assay was carried out by incubating the remaining extract (20 µl) with 500 ml 2 mM tyrosine in sodium phosphate buffer for 2 h at 30°C and absorbance was read at 420 nm. The whole experiment was repeated thrice. Activity of PO after 12 and 24 h of feeding on healthy mice was found to be more or less similar, but the activity at 18 h post feeding was significantly higher. On the other hand, PO activity in mosquitoes fed on *Plasmodium* infected blood, showed significant increase with time (Fig. 1.16) which indicated the probable role of PO in the immune response of mosquitoes against invading foreign body. Comparative immune response studies involving species A, B and C of the Culicifacies Complex are in progress.

1.4.3 Biochemical and molecular characterisation of Nitric Oxide synthase in *An. culicifacies*: relevance for refractory mechanism

The drive to identify novel control strategies has, in part, focused on identifying mosquito gene products that impart refractory phenotypes. Our goal is to develop tools for altering the vector competence of *An. culicifacies* which requires understanding the mechanism of vectorial resistance to the malaria parasite including biochemical and molecular studies of vector-parasite interactions. We have planned to use AcNOS (*An. culicifacies* Nitric Oxide Synthase) responses in mosquito vectors to *Plasmodium* as a tool to explore critical components of parasite development in mosquitoes and correlate it to the mechanism of refractoriness.

Specific activities of AcNOS (sp. B) in lysates of non-blood fed, blood fed, uninfected or *P. falciparum* infected mosquitoes at 6 and 9 days post blood meal activity were measured with or without NOS inhibitor L-NAME (1mM). Difference in AcNOs specific activities was much higher in infected and uninfected mosquitoes on Day 9 than on Day 6 in

Table 1.13. AsNOS and AcNOS specific activity in uninfected and infected mosquitoes gut samples

Sample	*An. stephensi*		*An. culicifacies* A		*An. culicifacies* B	
	Control	L-NAME	Control	L-NAME	Control	L-NAME
Non-blood fed	38.4	13.7	35.8	17.9	36.5	16.8
Day 6 infected	14.4	16.3	18.6	13.7	18.9	12.4
Day 6 uninfected	9.9	10.0	12.6	11.9	13.2	11.0
Day 9 infected	66.0	20.3	68.2	18.6	93.3	30.9
Day 9 uninfected	12.4	8.4	14.8	10.3	25.9	17.8

This data indicate significant differences at Day 6 and 9 parasite infection of NOS activity in control reactions. L-NAME (N-nitro-L-arginine methyl ester) inhibited in the infected mosquitoes guts.

Table 1.14. Primer details (Exon size, features , sequence, etc)

Exon no.	Exon size (bp)	Features	Amplimer size (bp)	Sequence(5'–3')
1	402	Haeme	200	ATGAGGACCAACTATCGGG GCCTTGGTGACAATGCTC
7	183	Haeme	163	AAGATGGGACTGGACACGC TGGTTTCGTTCTCAAAGTGC
15	170	FAD Ppi	162	GTGTCTACAAATCTTGGGAACC GCATCAGAAGCCTTTCCTCA
17	138	FAD NADPH	108	ATGGCTCTTCTTTGGCTGTC CGTGAAAGTGCCAGGAAAAC

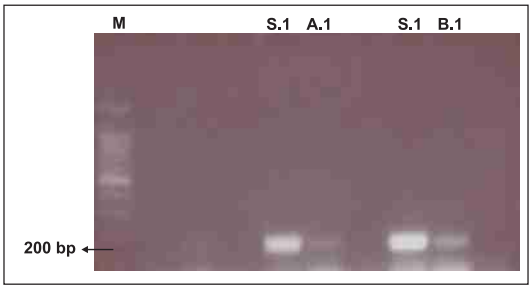


Fig. 1.17: PCR amplification of NOS gene using Primer 1 and 2 in *An. culicifacies* species B

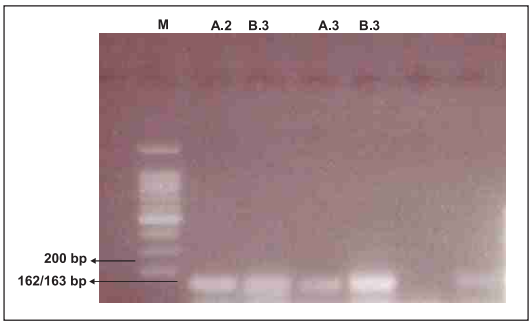


Fig. 1.19: PCR amplification of NOS gene using Primer 2 and 3 in *An. culicifacies* species A and B

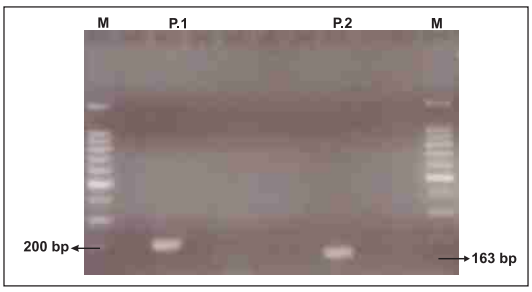


Fig. 1.18: PCR amplification of NOS gene in *An. culicifaces* species A and B using Primer 1

control reactions. The effect of L-NAME on AsNOS activity in infected mosquitoes was relatively unchanged by L-NAME at 6 days, whereas activity in infected mosquitoes was significantly inhibited by the inhibitor three days later on 9 Day (Table 1.13).

Four Primers were designed complementary to the *An. stephensi* exonic regions 1, 7, 15 and 17 encoding for the co-factors haeme, FAD PPi, FAD NADPH respectively (Table 1.14). Three primers P1, P2 and P3 primers have shown the amplification of the NOS gene by PCR (Figs. 1.17 and 1.18). Amplification of 200 base pairs against Exon 1 and Exon 7 and 15 have also shown the amplification of 163 base pairs (Figs. 1.18 and 1.19). This understanding is critical in evaluating the potential for manipulating *Anopheles* NOS gene expression to generate a refractory phenotype and this work will also reveal novel aspects of *Plasmodium* physiology for transmission blocking strategies.

2

Parasite Biology

2.1 Studies on genetic structure of human malaria parasites

2.1.1 *Plasmodium falciparum*

Plasmodium falciparum, the deadly parasite species is showing an increasing trend in India. Therefore, a study has been taken up to understand the population structure of the field isolates. Isolates from different geographical regions were analysed for surface protein markers—MSP-1 and MSP-2 for length as well as sequence variations. Analyses revealed highly polymorphic nature of Indian *P. falciparum* isolates on the basis of length as well

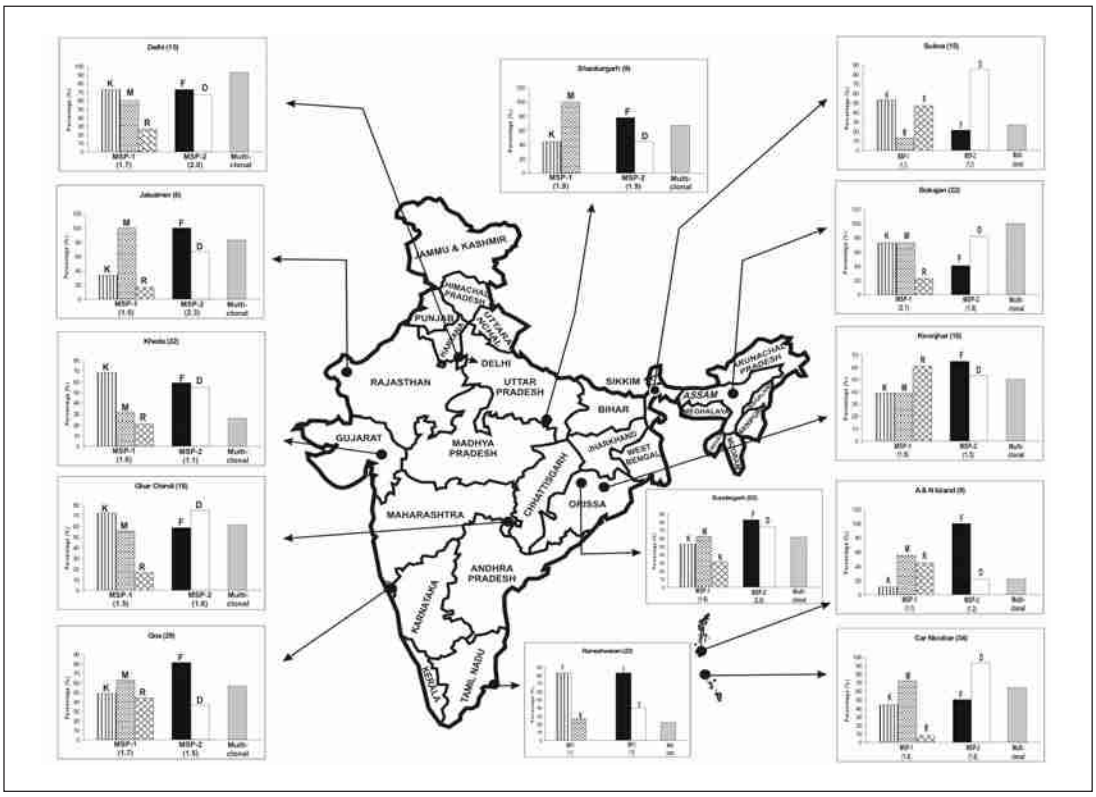


Fig. 2.1: *P. falciparum*: distribution of MSP-1 & 2 families among field isolates in India

as sequence data at these two loci. Three families of MSP-1 (K1, MAD20 & RO33) are prevalent in majority of the study sites. K1 and MAD20 families have shown allelic polymorphism while RO33 family was observed to be monomorphic with a single allele of 160 bp length in all the study areas.

In MSP-2, two families FC27 and 3D7 were observed in all the study sites and both the families have shown length variations. Fig. 2.1 shows the proportional prevalence of various families of MSP-1 and MSP-2. Sequence analysis of various alleles of MSP-1 and MSP-2 families revealed that Indian *P. falciparum* isolates present a mixed allelic composition representing different global regions. Sequences obtained have been submitted to Genbank.

Drug resistant markers

Field isolates from Rameswaram (Tamil Nadu), Panna (Madhya Pradesh), Sonapur (Assam), Sundargarh (Orissa) and Udaipur (Rajasthan) have been analysed for mutations in *pfcrt* (*P. falciparum* chloroquine resistance transporter) genes which are known to be associated with chloroquine resistance. Analysis revealed a high frequency of CQ resistant associated mutation (K76T) in *pfcrt* gene among the Indian field isolates. Fig. 2.2 shows the observed proportion of isolates showing K76T mutation in *pfcrt* gene among Indian *P. falciparum* isolates.

2.1.2 *Plasmodium vivax*

P. vivax, the most virulent species of malaria parasite in India that contributes about 55% of the total malaria positive cases in the country is responsible for huge burden of morbidity. Therefore, with an aim to understand extent of genetic diversity existing among the species, field isolates collected from different regions of the country with different eco-epidemiological conditions were analysed for polymorphism in genomic sequence by PCR, RFLP and sequencing. Following marker genes were used for the study.

GAM-1 (Gene coding for transmission blocking antigen)

Field isolates analysed from different geographical regions of the country such as Delhi, Tamil Nadu, Goa, Car Nicobar, Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar, Maharashtra and Orissa revealed dimorphic nature of GAM-1 among Indian isolates. Both Belem and Chession type alleles were present with prevalence of Belem type allele (above 70%) in all the sites studied except in Orissa. In Orissa isolates Chession allele was predominant (about 80%) which is similar to Thailand isolates, while isolates of other areas showed resemblance to Korean isolates. On the other hand, Indian isolates showed a totally different picture from Sri Lankan

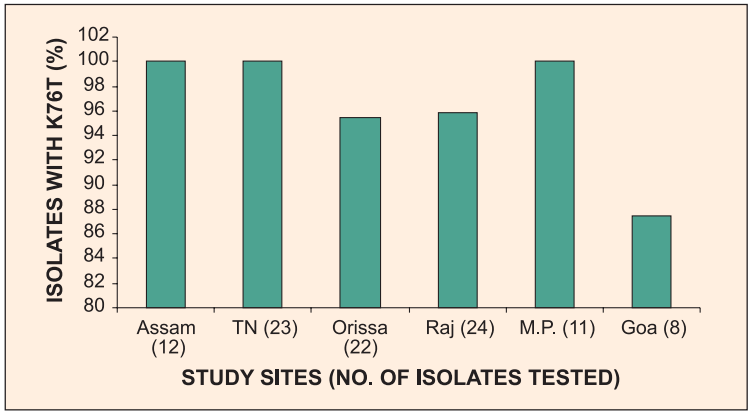


Fig. 2.2: Proportional prevalence of *pfcrt* K76T mutation among Indian *P. falciparum* field isolates

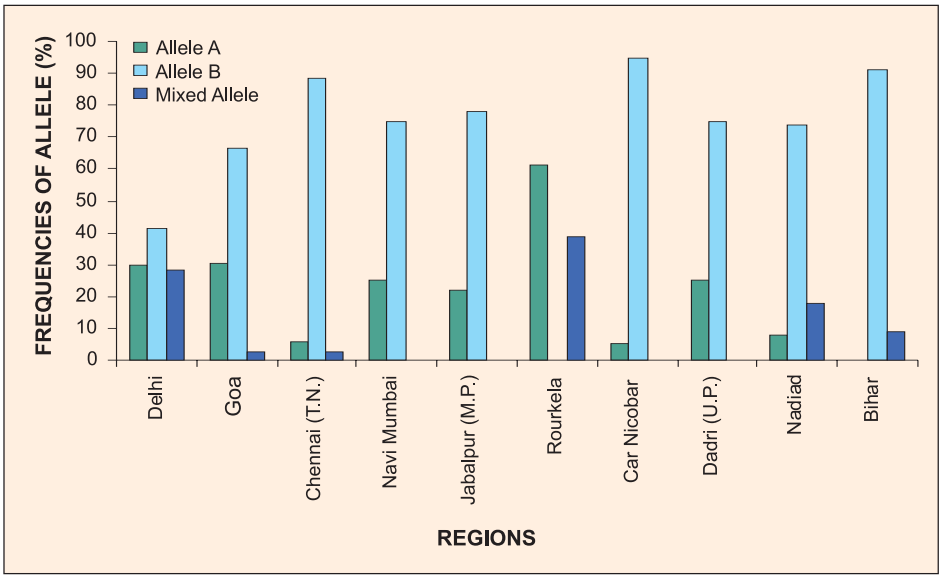


Fig. 2.3: Distribution of *P. vivax* GAM-1 alleles in different parts of India. Allele A–Chession type and Allele B–Belem type. Mix allele – presence of both Belem and Chession type together in the isolates

isolates which were high polymorphic. Fig. 2.3 shows the proportional prevalence of two alleles (exclusively or on combination) among the Indian isolates.

MSP-3α

PCR revealed three different fragment sizes (length variants) with predominance of 1.8 kb fragment (above 75%) in isolates studied from different regions such as Delhi, Tamil Nadu, Goa, Car Nicobar, Gujarat, Madhya Pradesh, Maharashtra and Orissa. RFLP with Alu I and Hha I revealed a total of 19 different alleles among Indian isolates compared to 13 isolates reported from Papua New Guinea (PNG) and Thailand. Sequence analysis of the PCR amplified fragments (all 3 variants 1.8, 1.4 & 1.2 kb) revealed that 1.8 kb variant resembles closely to Korean and Thailand data while 1.4 kb variant resembles more to Venezuela data and 1.2 kb variant is like Chession strain. Study further revealed block-I region of MSP-3α was deleted in 1.2 and 1.4 kb fragments, in addition 1.2 kb variant α-helix was also deleted thus creating a high degree of diversity among the isolates. Fig. 2.4 gives the distribution of three size variants of MSP-3α among Indian isolates.

Pv SSUrRNA – Two sub-types

It is believed that *P. vivax* from the new world should be considered a separate subspecies to that present in the old world. In order to confirm/reject the hypothesis, we analysed the 18S SSUrRNA S-type gene. Isolates from different regions of India were analysed and the results revealed the presence of both old and new world type S-gene of SSUrRNA among the isolates with an even split of 60% old world and 40% new world type. Similarly, isolates from PNG and Brazil also showed presence of both the types. In PNG isolates predominance of old world type (90%) was observed while among Brazil isolates predominance of new world type (70%) was observed. Above observation suggested the presence of both the S-type allele frequencies worldwide clearly demonstrating the global distribution of the two alleles and no geographical subdivision. Fig. 2.5 shows the prevalence of old and new world S-type allele of SSUrRNA among isolates of India and other global regions.

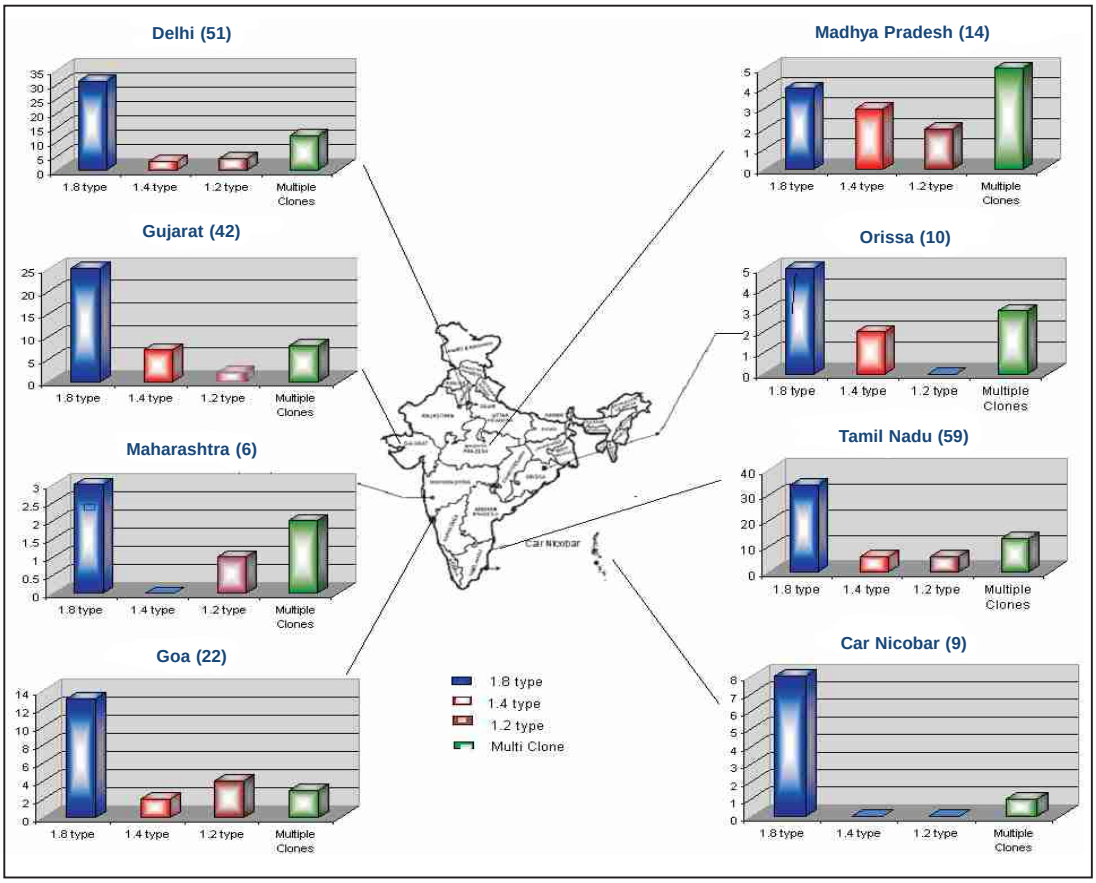


Fig. 2.4: Allelic distribution of MSP-3α in India

2.2 Development of nuclear DNA markers for evolutionary studies in *P. falciparum*

Estimation of genetic diversity in species populations and inferring evolutionary dynamics of different genes are important in biomedical research, especially in finding new drug target genes and developing new effective drugs and vaccines. Recent researches in evolutionary genetics have revealed that estimation of genetic diversity is strongly dependant on the genetic markers used, thus making appropriate evolutionary inference both at species and gene level, difficult. Considering these facts, we have developed nuclear DNA markers in human malaria parasite—*P. falciparum* using the published whole genome sequence information. Specifically we follow the approach described recently in *Drosophila* to analyse genetic fragments that are located in introns (non-coding sequences) to infer the demographic history of populations. Such fragments are amplified by PCR technique by designing primers in the flaking exons (coding DNA sequences). Following this approach that is commonly known as EPIC (exon priming intron crossing) fragments, we developed a total of 170 putatively neutral fragments in the whole genome of *P. falciparum* (Fig. 2.6). Likewise, in order to detect endemic mutations in different alleles in the *pfcrt* gene and measure genetic diversity

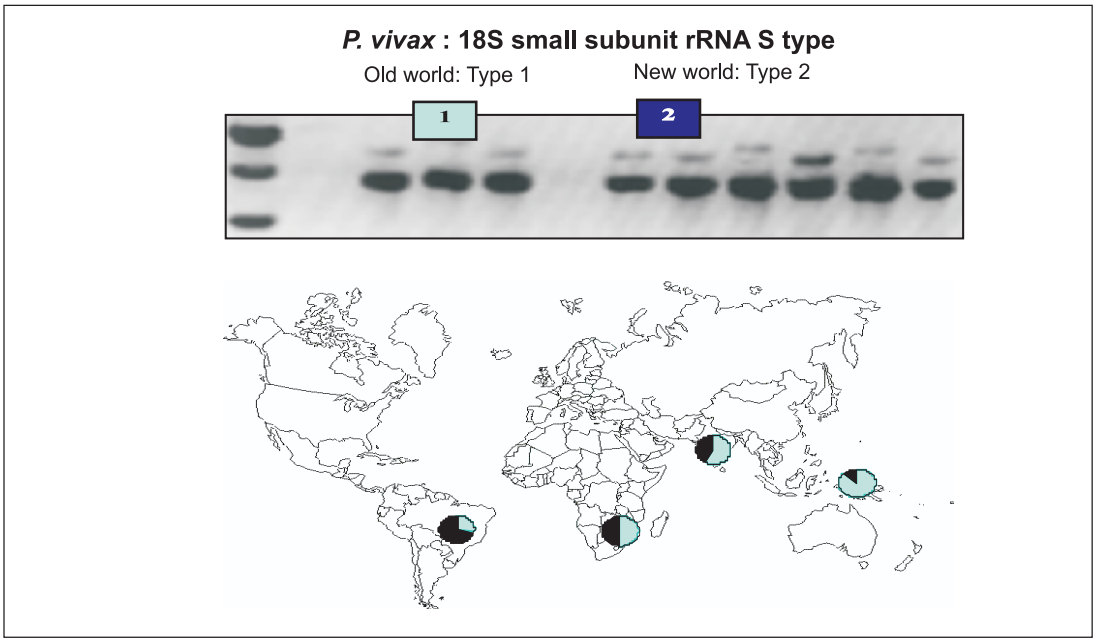


Fig. 2.5: Global distribution of old and new world S-type allele of 18S SSU rRNA

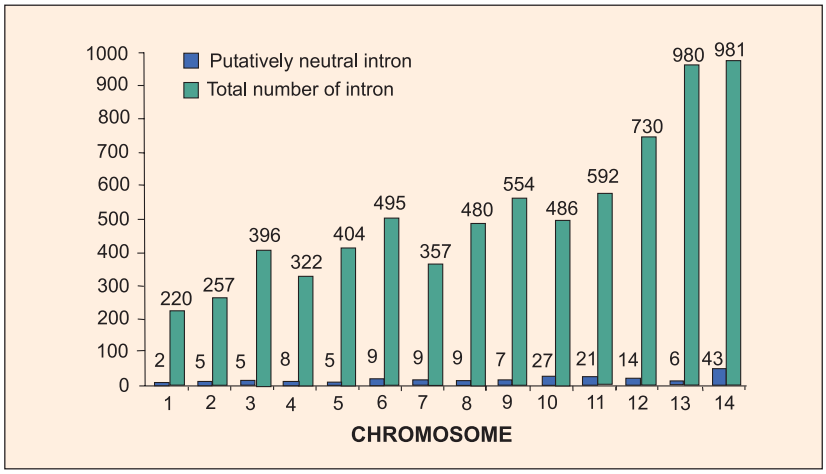


Fig. 2.6: Putative neutral introns in *P. falciparum*

in both the resistant and sensitive varieties in isolates collected in different geographical populations in India, we intend to sequence the whole *pfcrt* gene, instead of looking at the specific local mutations. We designed three primary primer pairs for amplifying the whole *pfcrt* gene and five nested primer pairs for sequencing the whole gene in short stretches (Fig. 2.7). Considering the drug pressure in the field

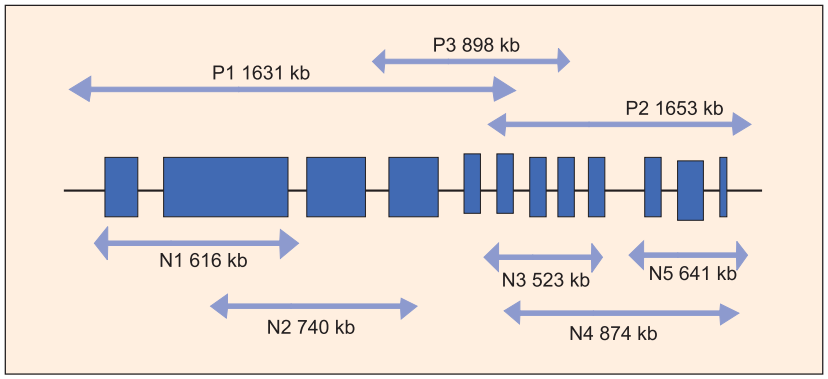


Fig. 2.7: Primary primers for amplifying *pfcrt* gene and whole gene in *P. falciparum*

and reported sweeping of the resistant allele of the *pfcrt* gene by natural selection, we are interested to know the detailed evolutionary history of this gene in Indian *P. falciparum*. The development of nuclear DNA marker would thus help in understanding the precise roles of demography and natural selection in evolution of *P. falciparum* in India.

2.3 Evolution of introns in the genome: inference from the whole genome scanning of the human malaria parasite *P. falciparum*

The *P. falciparum* genome is unique in several aspects. The long proteins compared to homologous in other organisms, high AT rich genome (80.6%), etc. are some of the important observations from different studies. In this context, a genome-wide survey and study of *P. falciparum* for understanding of genome organisation might point towards the unique characteristics of the species. The comprehensive data sets available in public databases such as PlasmoDB and NCBI combined with the availability of substantial sequence tracts from *P. falciparum* has made it possible to embrace this genome wide study (Fig. 2.8). It has become clear that introns have been gained and lost in different lineages at various rates. In this context it is of particular interest to estimate

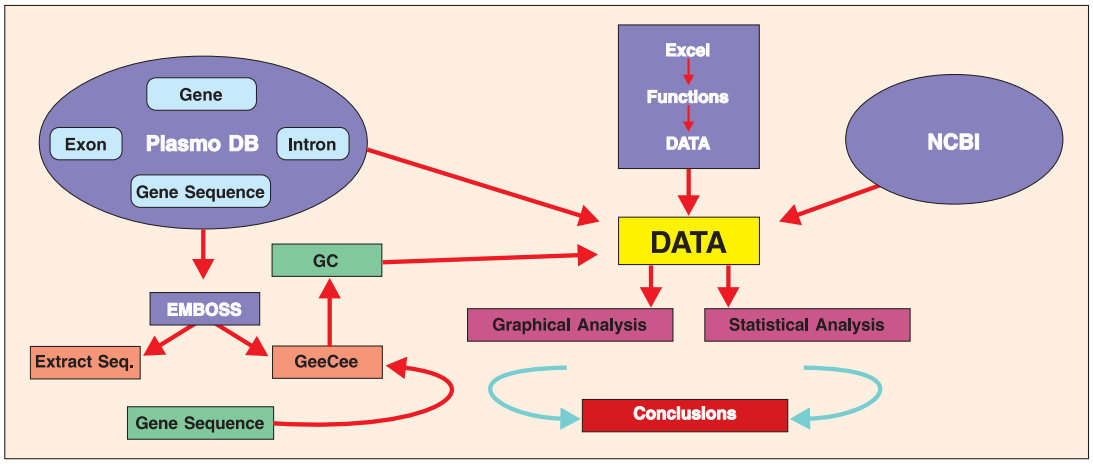


Fig. 2.8: Schematic representation of data collection and processing

the intron densities in lower eukaryotes, such as *Plasmodium*.

We followed a defined method (Fig. 2.8) for data retrieval from the web data source (PlasmoDB and EMBL) and collected according to our analytical need. We found most genes were small but the hypothetical genes were comparatively larger than the genomic average. However, functional genes were small in general and clustered in the sub-telomeric regions of the chromosome. Introns in the functional genes were found to be smaller than the average genomic length and small genes were considerably rich in GC content. The number of genes on each chromosome was marginally correlated to chromosome size. A statistically significant positive correlation (Pearson's $r = 0.92$, $p < 0.001$) was detected between the number of introns and chromosome size. A statistically significant positive correlation ($r = 0.93$, $p < 0.001$) between the number of introns and number of genes along each chromosome was also observed. Generally, the number of introns was found to increase in the increasing order of the chromosome size. However, this pattern was found to be distorted for the two largest chromosomes, chromosome 13 and 14 (Fig. 2.9). As evident in the figure, very large sized introns were found in chromosome 14, the largest of all chromosomes.

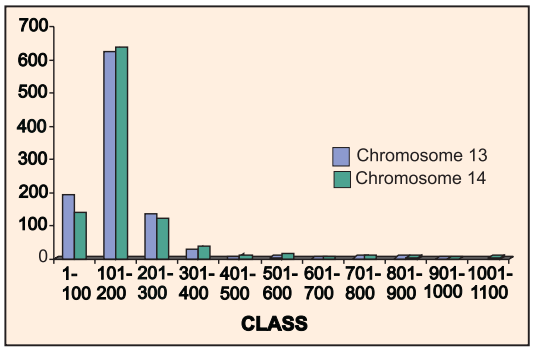


Fig. 2.9: Intron frequency table based on size in chromosomes 13 and 14

The other observed features in the present study, like conservation of GT-AG splice site, presence of polypyramidine tail at 3′ end, etc. could throw light to the theories suggesting the invasion of introns in eukaryotic genome after the separation from other lineages as suggested by intron late theory. The highly AT rich introns detected in this study seems to contribute much to the overall AT based nature of *P. falciparum* genome. Most genes are intron-less and fall in the size range of 100-200 bp in length.

2.4 Identifying single clonal infections from the archived sample

Blood spots collected on Whatman 3 mm filter paper strips from microscopically positive *P. falciparum* subjects and those available in NIMR archive were analysed for the presence of diverse MSP-1&2 and GLURP alleles for identification of isolates having single clone of *P. falciparum*. Ninety single clone isolates have been identified from Orissa, Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Rajasthan and Gujarat.

2.5 Development of website on the genetic and biological diversity of Indian *Plasmodium*

Workers in the field have already documented enormous genetic and biological diversity in malaria parasites in India and more studies are pouring in reporting wide diversities in both the characteristics of the parasites. However, there is a strict lack of comprehensive information on the biological and genetic diversity of Indian species of *Plasmodium*. In view of this, a webpage containing comprehensive information on the biological and genetic diversity of Indian Plasmodia has been developed. This webpage would also contain information on the workers actively involved in this field. The website can be accessed from www.plasmodiversity.org.in. The beta version of the website is already been released and the screenshot of the website is depicted in Fig. 2.10.

2.6 Expression of T-helper cell epitopic regions of circumsporozoite protein (CSP) of *P. falciparum* in *Escherichia coli*

Importance of T-cells in malaria immunity has been appreciated for long. Two T-helper cell epitopes have been located at the C-terminal end flanking, the

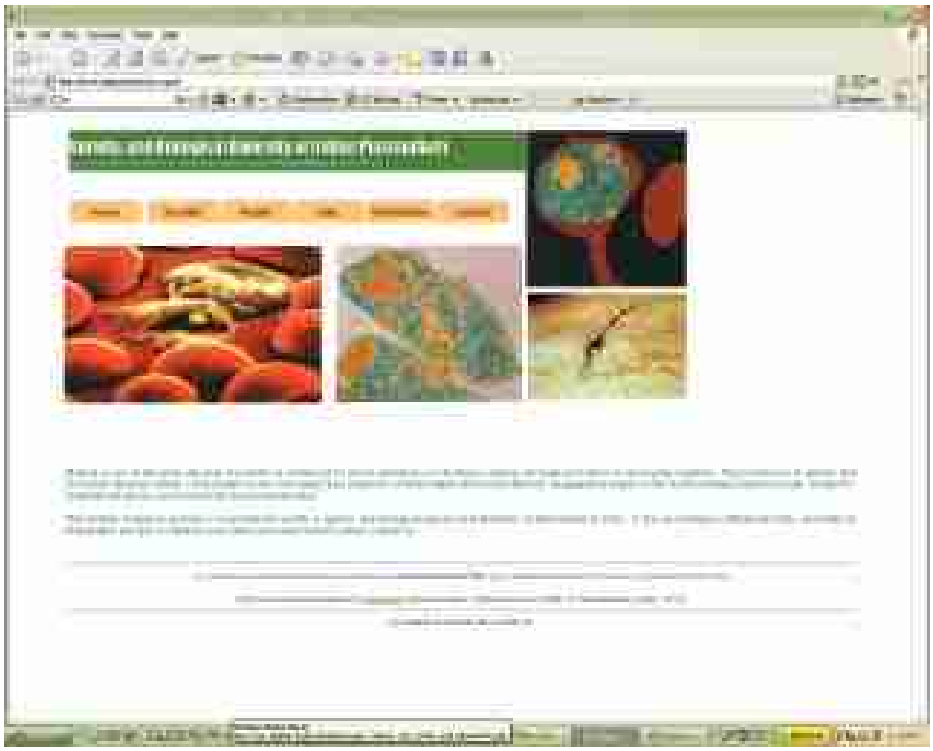


Fig. 2.10: Website on genetic and biological diversity of Indian *Plasmodium*

highly conserved region RII of CSP. Th-epitopes show variation. Even then if the variations are restricted and can be grouped, then the prototype variants from the group could be included into subunit polyvalent vaccine against sporozoites. The genetic variation in Th-epitopes of 148 *P. falciparum* isolates collected from different epidemic and endemic regions of India have been studied. Variations have been found to be regionally unbiased in the sense that similar type of variation has been found in different regions. The allelic variants could be categorised into 4 groups. Since the number of variants is small, then the prototype variants could be included into a subunit polyvalent vaccine against sporozoites.

The prototype variants have been cloned into Pqe-40 expression vector and expressed it as a fusion protein fused to DHFR (Dihydrofolate reductase) gene tagged to 6 His residue. Fusion protein has been purified and the study on the immunogenicity of fusion protein is in progress.

2.7 Relatively simple genotype of *P. falciparum* isolates from India as determined by anchored primer amplification of DNA

Genotyping of *P. falciparum* is important for therapeutic efficacy and other purposes. Various methods have been developed for genotyping of malaria parasite especially for *P. falciparum*, but they have their limitations. The poly A and poly T using oligonucleotides anchored at the 32 end of the primer, the genetic differences in the AT rich DNA sequences can be displayed. Genomic DNA of *P. falciparum* isolates was prepared from the blood samples collected from both endemic and epidemic areas of India. This genomic DNA was PCR ampli-

fied using single anchored poly A or poly T primers. With dinucleotide anchored primer in most of the cases, smear was obtained, even at increased concentrations of $MgCl_2$ whereas with tri, tetra and penta nucleotide anchored primers, though different patterns of bands were observed, they were less complex compared to the bands seen in the study. Thus, it can be concluded that the genotype of Indian *P. falciparum* is more simple than that of the isolates from other geographical regions of the world.

2.8 Association of chloroquine resistant molecular markers in *P. falciparum* isolates from India

Chloroquine (CQ) is the most widely used drug for the treatment of malaria, but development of CQ-resistant parasites has made both treatment and control of malaria complicated. Mutational changes in three genes, *pfmdr1*, *pfcrt* and *cg2* have been attributed to chloroquine resistance. However, alleles of a single gene could not be accounted for CQ-resistance. An association of alleles of these genes is believed to be responsible for CQ-resistance. Twenty CQ-resistant and 20 CQ-sensitive *P. falciparum* isolates from different geographical regions of India have been studied to determine the association of CQ-resistant molecular markers. Genomic DNA was prepared from 20 CQ-resistant and 20 CQ-sensitive isolates. The portion of the gene, harbouring mutational site was PCR amplified using primers flanking the mutational sites. PCR amplified products were purified and sequenced. It has been found that some of the resistant strains contain the *pfmdr1* mutation N86Y, while others did not. Same is the situation with *pfcrt* K76T. Thus, it appears that mutation in any single gene is not responsible for CQ-resistance, but together they might confer CQ-resistance. However, the role of cg2 gene is ambiguous. Therefore, it can be concluded that, association of mutation in *pfmdr1* and *pfcrt* might be responsible for CQ-resistance.

2.9 Serum and immunoglobulin mediated inhibition of intraerythrocytic growth of *P. falciparum in vitro*

A study on malaria parasite profile and acquired immune response to *P. falciparum* stage specific proteins was conducted in subjects reported with fever in eight different areas of the country, where malaria is seasonal. Both *P. vivax* and *P. falciparum* infections may occur in population during natural course of infection of malaria. This study was undertaken with the following objectives: (i) to measure the level of antibodies developed during natural course of malaria infection in individuals of various age groups of the study areas of different endemic situations, (ii) to analyse the effect of immune pressure exerted by antibody on *P. falciparum* parasites by sera and immunoglobulins mediated growth inhibition *in vitro*, and (iii) to determine the quantitative measurement of parasite invasion with variable level of antimalarial antibodies as a result of selecting subpopulation of parasites *in vivo* for maintaining parasite infection below threshold.

One hundred thirty-eight patients of eight study areas were included in the study. These 138 patients were categorised into five epidemiological age groups, such as: 1–<5; 5–<10; 10–<15; 15–<25 and >25 yr. *P. vivax* was detected in 49 (35.5%) and *P. falciparum* in 33.3% patients. Finger prick blood samples were collected by cross-sectional surveys during July to November of the years 2000–04, from all 138 patients; those were reported with malarial symptoms, especially fever. Some patients had earlier history of malaria but found malaria parasite negative at the time of blood collection. Thick and thin smears were examined after staining with JSB stain. Parasite density was estimated by counting the number of parasites per 200 leucocytes and was converted to number of parasites/µl blood taking 8,000 leucocytes/µl as standard. After collection of blood samples, patients were treated with antimalarial drug as per national treatment policy for malaria in India.

All the sera were tested for antimalarial antibody by ELISA against defined peptides and crude erythrocytic antigens of *P. falciparum*. Sera of these patients demonstrated low, moderate and high levels of antibodies against Pf-stage specific antigens—MSP-1, AMA1, RAP1, EBA175 and infected erythrocyte lysate (*Pf* crude antigen). The effect of antibody positive pooled serum and immunoglobulin fractions

with high ELISA O.D. values was assessed in *P. falciparum* culture by determining the merozoite invasion inhibition (MII) and parasite total growth inhibition (PGI). The sera of responder group containing antibodies showed variable effect in parasite growth of two *P. falciparum* isolates (Figs. 2.11 and 2.12). A positive correlation between MII and PGI of two isolates has been observed. Sera containing high titre of antibodies reacted efficiently in immunofluorescence assay. Reactivity was more pronounced with late trophozoite and schizont stages. Similarly, pooled antibody positive sera showed low to high intensity of colour development in dot blot assay.

Malaria morbidity and mortality in human populations vary greatly under different circum-

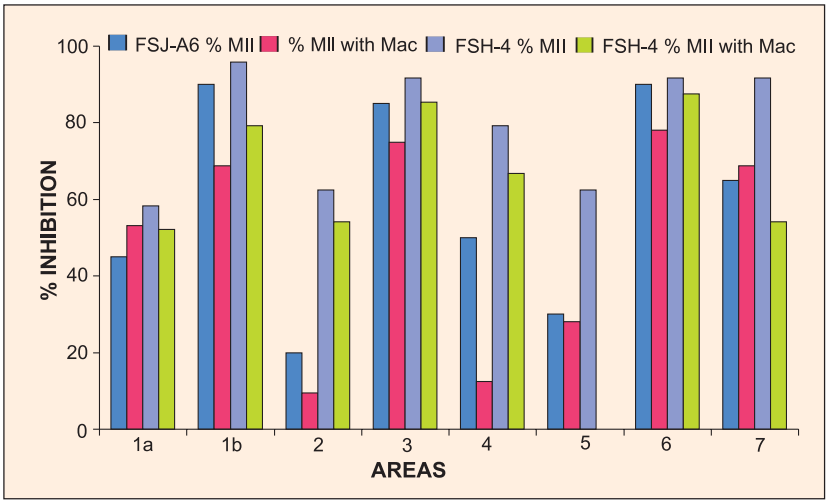


Fig. 2.11: Effect of serum in *P. falciparum* growth *in vitro*

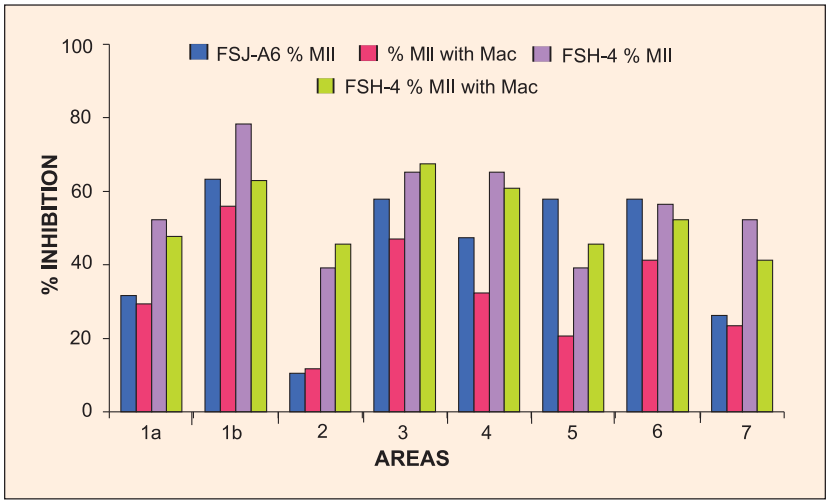


Fig. 2.12: Effect of immunoglobulin in *P. falciparum* growth *in vitro*

stances. With the attainment of age and repeated exposures to malaria infection, immunity builds up and it results in decline of malaria incidence and also clinical tolerance in adults. Inhabitants of malaria endemic areas in general possess antimalarial antibodies in circulation. In endemic areas, there are still some healthy populations who remain free from malaria due to their innate immunity against malaria.

Human serum varies considerably in its ability to support parasite growth. The specific antibodies may play an important role in mediating protective immunity and it is probable that these antibodies require the cooperation of sensitised effector cells to achieve parasite destruction *in vivo*. Human beings, who are semi-immune because of chronic and repeated infections with *P. falciparum*, may also possess cross-reactive immunity to some strains of *P. falciparum*.

The sera from various endemic zones are able to select parasite subpopulation based on their specific neutralising activities. Since inhibition of parasite growth by antibody-mediated merozoite agglutination *in vitro* may reflect an important mechanism of protective immunity, thus characterisation of *P. falciparum* for antigenic diversity within and in between geographic areas using those sera might be helpful in selecting strains for inclusion in malaria vaccine strategy.

2.10 Raising antibodies against synthetic peptides of PfHRP-2 and pLDH using microsphere delivery: Development of diagnostic reagents

Antigen tests are promising tools for the diagnosis of malaria. Two such antigens are *P. falciparum* histidine rich protein (PfHRP-2) and lactate dehydrogenase (pLDH). PfHRP-2 is water soluble protein released from parasitised erythrocytes into *in vitro* culture supernatants; pLDH, a glycolytic pathway enzyme of the malarial parasite is produced by sexual and asexual stages and can be detected in culture supernatant and plasma of infected patients. The present study was aimed to develop indigenous, rapid and sensitive immunodiagnostic method based on the detection of PfHRP-2 and pLDH antigens in the blood. Unique peptide sequences of PfHRP-2 (two regions) and pLDH (three regions) antigens were synthesised by solid phase technique and purified to homogeneity. The antibodies against these sequences were raised in mice as well as rabbit using microspheres (PLGA)

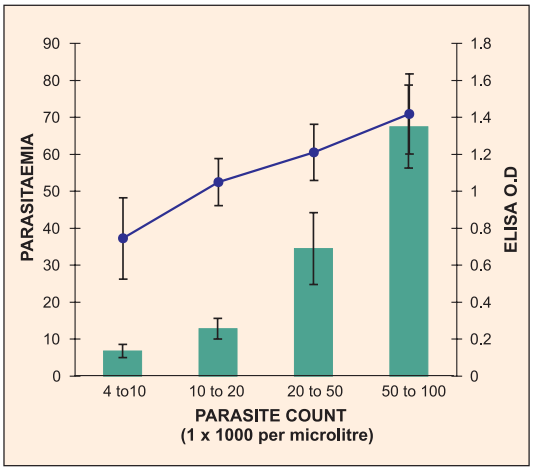


Fig. 2.13: Relationship between mean parasitaemia (parasites/µl) (■) and mean HRP-2 antigen concentration (-●-) in patients of *P. falciparum* malaria

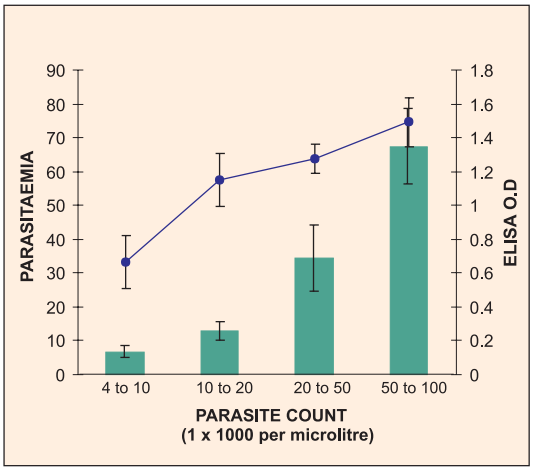


Fig. 2.14: Relationship between mean parasitaemia (parasites/µl) (■) and mean LDH antigen concentration (-●-) in patients of *P. falciparum* malaria

to generate high titre and affinity antibodies. The peptide specific peak titres varied from 25,000–50,000 and affinity of the antibodies produced was found to be in order of 0.73–5.3 nM. The antibodies generated using microspheres were able to detect the PfHRP-2 and pLDH antigens in the culture supernatant and parasitised RBC lysate of *P. falciparum* respectively by sandwich ELISA up to 0.002% parasitaemia level (Figs. 2.13 and 2.14). The assay allowed the detection of parasite infections of 0.08–2.68% parasitae-mia with a sensitivity of 100% in the whole blood of *P. falciparum* positive patients. No cross-reactivity was observed with *P. vivax* infected patients.

2.11 Detection of circulating antigen, antibody and immune complexes in individuals of endemic area as immunometric parameters in malaria

Human blood samples collected from local malaria clinics, hospitals and by cross-sectional surveys in malaria endemic areas were tested by enzyme immunoassay for circulating malarial antigens, antimalarial antibodies and antigen-specific circulating immune complexes. The assays were done in serum and finger-prick blood absorbed on filter paper. A total of 117 patients were screened for malarial parasite by microscopy; 54 were found positive and rest 63 were negative at the time of blood collection but they suffered from malaria in the recent past. The ELISA tests for the detection of antimalarial antibody, circulating antigen and circulating immune complexes were done in 117 sera and FP eluates as a pilot study. The results of sera and FP eluates were comparable. Out of 54 malaria positive samples, 51 and 52 were detected positive for FC-Ag in sera and FP eluates, respectively. It shows that the test has high diagnostic efficiency. Only 25–28% samples of negative group were detected FC-Ag positive due to very recent infection in these individuals. Antibody was detected in > 90% malaria patients whereas in blood smear negative group about 86% showed sero-positivity for antimalarial antibody. The bound antigens in the form of CICs were detected more in malaria positive cases than those with smear negative. The test parameters were applied in 240 FP eluates collected from different age groups (43, 60, 56 and 81 aged 1–<5, 5–<10, 10–<15 and >15 years, respectively). The average antibody titre $-\log_2$ varied from 6–10. Both TC-Ag and CIC were found at lower level in younger age groups (Fig. 2.15). Although microscopic demonstration of malarial parasites in blood films is the method of choice to

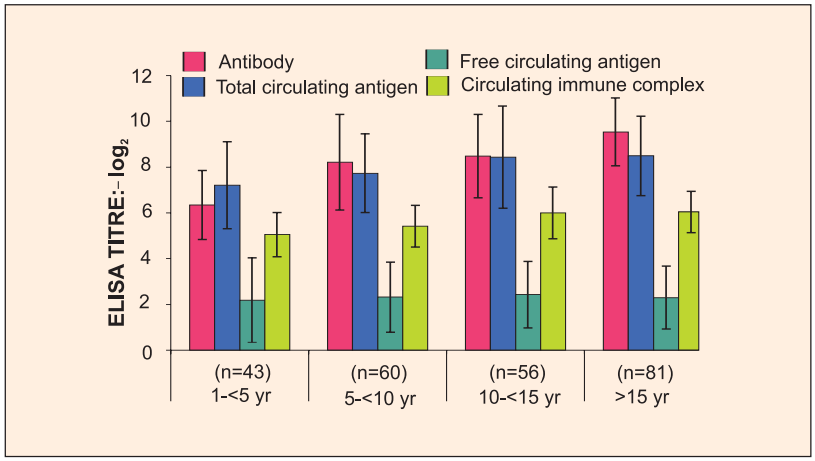


Fig. 2.15: Antibody, total circulating antigen, free circulating antigen and circulating immune complex profile in the study subjects (n = 240). The bar diagram is plotted with mean ELISA -$\log_2$ titre of each group

diagnose acute malaria, detection of circulating malarial antigens, antimalarial antibodies and immune complexes may help as supplementary tool for immunodiagnosis. When applied in a community-based study, these tests would be able to monitor infection dynamics.

2.12 Immune responses to *P. falciparum* antigens and disease susceptibility among inhabitants seasonally exposed to malaria

In malaria endemic areas, the acquisition of antimalarial immunity is progressive as observed by age wise gradual decrease in morbidity and mortality to malarial infection. This study was aimed to assess the relationship between *in vitro* immune responses and susceptibility to malaria based on a protective longitudinal study for one year.

In the present study, two groups of children, younger (1–<5 yr) and older (5–<15 yr) were enrolled in two areas (A-1 and A-2) of northern India where malaria is though seasonal but the epidemiology is different. The frequency of humoral and cellular reactivity to six synthetic peptides (CSP, MSP-1_{19}, AMA1, RAP1, EBA175 and PfG27) and crude blood stage antigens of *P. falciparum* were measured in the study subjects.

Individuals of A-1 showed very less number of malarial episodes compared to A-2 during one year. Clinical protection in children of A-1 was related to elevated levels of antibodies detected against CSP, MSP-1_{19}, AMA1 and RAP1. However, anti-EBA175 antibody level was almost alike in two groups. Higher lymphocyte proliferation responses to MSP-1_{19}, AMA1 and RAP1 peptides were observed in the study subjects of A-1 than A-2. The differences in T-cell reactivity as overall higher response rate of IL-4 and IFN-γ but lower IL-10 and TNF-α to MSP-1_{19}, AMA1 and RAP1 were observed in individuals of two areas. The immune responses among individuals of two ecotypes highlight the immunogenicity of the molecules, namely MSP-1_{19}, AMA1 and RAP1 and their relation to clinical protection.

2.13 Molecular characterisation of aspartic protease gene from *P. vivax*

Aspartic proteases from *P. falciparum*, known as plasmepsins have been characterised and are believed to have a promising potential in antimalarial chemotherapy. Aspartic proteases from *P. vivax* have been characterised. Characterisation at the biochemical and molecular level will be helpful in designing new drugs against *P. vivax* and *P. falciparum*. As an extension of the previous work, a study was planned to characterise Aspartic protease gene in *P. vivax* with the following objectives: (i) N-terminal sequencing to confirm the homology to known Aspartic protease of *P. falciparum;* (ii) to find out the difference among the samples collected from different geographical regions (different forms of plasmepsins namely I, II, IV, etc.); (iii) to characterise the Aspartic protease genes from *P. vivax;* (iv) to clone and express the gene of Aspartic protease from *P. vivax* and compare with that of *P. falciparum;* (v) to confirm activity of recombinant protein equivalent to native protein using conformational/ biochemical analysis; and (vi) use of recombinant enzymes for kinetic analysis of inhibitors/drug designing for drug target.

This study will be helpful to elucidate the biochemical properties and biological role of malarial proteases and will foster the development of protease inhibitors as a new antimalarial drug. This rational drug design will help in targetting common susceptible sites of both *P. falciparum* and *P. vivax* and may prove to be a boon for the antimalarial chemotherapy.

2.14 Glutathione S-transferase (GST) activity: diagnostic and protective role in *vivax* malaria

Glutathione S-transferase is the most abundant intracellular antioxidant with complex biological functions and a well-established metabolic regulator. In malarial infection, host produces oxidative stress and maintains it as a defense mechanism. The Glutathione S-transferases play a critical role in malarial diagnosis and pathogenesis. The malarial parasite—*P. vivax* is known to be sensitive to oxidative stress,

Table 2.1. Comparison of serum and plasma GST activity in healthy and *P. vivax* infected patients

Subjects	GST (IU/L)		
	Minimum	Maximum	Mean ± SD
Serum			
Control	7.25	16.65	11.65 ± 2.95
P. vivax	2.6	15.6	6.43 ± 3.29
Plasma			
Control	6.15	13.23	10.09 ± 2.68
P. vivax	2.37	13.56	5.65 ± 3.15

and thus the antioxidant enzyme—GST has attracted interest in the field of diagnosis and monitoring in view of malarial complications.

With this objective in mind, we have collected 39 (32 Male/7 Female) clinical isolates of malaria patients infected with *P. vivax* and 9 (Male) healthy controls from clinic and analysed serum and plasma GST activity in *P. vivax* patients and healthy control essentially following the procedure described by Habig *et al** (Table 2.1).

Serum and plasma GST levels of *P. vivax* infected patients had less GST activity (6.43 ± 3.29 and 5.65 ± 3.15 IU/L) as compared to healthy controls (11.65 ± 2.95 and 10.09 ± 2.68 IU/L), ($p < 0.0004$) and ($p < 0.0007$). A significant correlation in the GST activity of control subjects and *P. vivax* patients (Fig. 2.16) was observed ($r = 0.95$). Further studies are in progress.

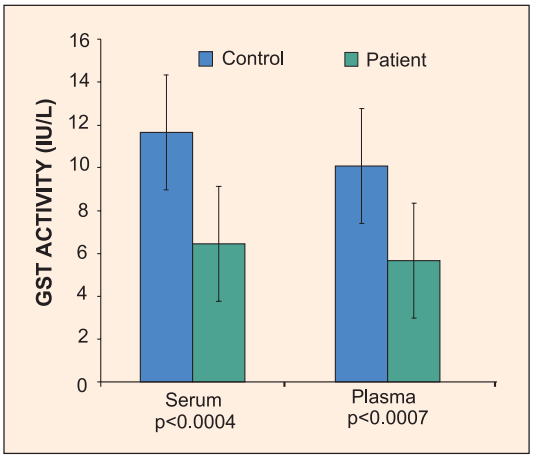


Fig. 2.16: Comparative glutathione S-transferases (GST) activity in plasma and serum in *vivax* patients and healthy controls

*Habig *et al* (1974). *J Biol Chem* **249**(22): 7130–7139.

2.15 Malaria Parasite Bank

Parasite Bank is supporting a large number of organisations working on various aspects of malaria. Biological material including non-human and human plasmodia preserved/maintained at the Malaria Parasite Bank were supplied to various research organisations. During 2004–05, 16 *P. falciparum* isolates adapted to *in vitro* culture conditions and characterised for drug susceptibility to CQ have been supplied to the Department of Biotechnology, AIIMS, New Delhi and another 30 *P. falciparum* isolates were supplied to Division of Molecular Biology, NIMR as part of collaborative studies. Twenty *P. vivax* isolates were given to ICGEB, New Delhi on payment. Human and/non-human malarial parasites were given to PGIMER, Chandigarh, JNU, New Delhi and University of Hyderabad, Hyderabad. As a part of resource generation we have already started charging for the biological materials supplied from the Parasite Bank and also for screening of medicinal plants for their antimalarial activity. During 2005–06, 14 *P. falciparum* and 5 *P. vivax* isolates from Mangalore, one *P. vivax* from Bangalore, 3 *P. falciparum* and 3 *P. vivax* isolates from Chennai were collected.

2.16 Screening for antimalarial activity of the synthetic compounds in *P. falciparum* culture lines

A number of compounds were synthesised by novel methods to study their antimalarial activity *in vitro* in *P. falciparum*. Some novel derivatives as six new Baylis-Hillman adducts were synthesised based on substituted 2-chloronicotinaldehydes and five 1-aryl-4,6-diamino-1,2-dihydrotriazines were synthesised using neat technology under microwaves. These compounds were tested *in vitro* against chloroquine and pyrimethamine sensitive and resistant *P. falciparum* strains.

2.17 Screening of medicinal plant extracts/ fractions for antiplasmodial activities

Screening of medicinal plant extracts/fractions for their antiplasmodial activity against chloroquine sensitive and resistant *P. falciparum* isolates is a routine activity of the Malaria Parasite Bank.

In the new collaborative project entitled, "Discovering antimalarials from marine organisms" (multi-institutional collaborative project with ICGEB and University of Kerala, funded by DBT) it was proposed to collect flora and fauna from marine and estuarine conditions for the preparation of extracts which is being done in University of Kerala. These extracts are being tested in ICGEB and NIMR for their antiplasmodial activity. We have already screened 32 marine samples for their antiplasmodial properties *in vitro* against CQ sensitive and resistant isolates of *P. falciparum* and few of them are showing very good anti-plasmodial activity. Screening of other samples are in progress.

Due to the resistance of parasites to almost all antimalarials available, efforts are being made to explore the possibility of new antimalarials from medicinal plants available indigenously (either in the crude form or molecules). Keeping this in mind, about 25 medicinal plant extracts were tested and some of them were showing good antiplasmodial activity *in vitro* against *P. falciparum*. Three extracts were tested *in vivo* also. These extracts showed up to 75% inhibition with 50 mg/kg body weight. Further work is in progress for the purification of compounds from these extracts.

Fifteen samples (medicinal plant extracts and pure compounds) from DRDE, Gwalior were tested for their antiplasmodial activity and few samples are being processed. Twenty-two marine samples were screened for antimalarial activity.

The collaborative project entitled, "Primary screening of the medicinal plants from Northeastern states of India for their antiplasmodial activity" is an ongoing project. Under this project 25 crude extracts and 8 fractions were tested *in vitro* till now. Out of these one crude extract tested *in vivo* showed about 60% inhibition with 100 mg/kg body weight. Further studies are in progress.

As a part of manpower development, parasite bank is actively involved in imparting training to research fellows/students in *in vitro* cultivation of *P. falciparum* and drug sensitivity testing. Also, as per request from University of Khartoum, Sudan, a workshop was conducted on identification and *in vitro* cultivation of malaria parasites, screening of antimalarials, establishment of malaria parasite bank, etc., in Sudan during 28 May to 12 June 2005. ❑

4

Highlights of Research Activities under IDVC Project

Bangalore (Karnataka)

WHO 28-day extended *in vivo* studies to detect chloroquine resistance against *P. falciparum* were carried out in PHC Narsapura, District Kolar and PHC Tavarkere, District Tumkur. A total of 71 *Pf* cases were successfully followed-up. All the 9 cases from Kolar were resistant to chloroquine and showed positive correlation to *Pfcrt* and *Pfmdr1* genes. Screening of 62 samples from Tumkur is under process. Lambdacyhalothrin 10 CS formulation against *An. culicifacies* was found very effective up to 12 weeks and was found safe to human beings. Situational analysis of malaria in three districts in Karnataka namely Kolar, Chitradurga and Raichur were carried out. Malaria in all the districts is under control. Mosquito control in Bangalore City and malaria control in Mangalore City were continued. It was found that malaria in Mangalore City was restricted to construction areas and needs administrative control over the building owners by the local administration. Malaria in all the areas where larvivorous fish were released is under control. Malaria outbreak investigation was carried out in Hassan and Tumkur districts and found that the cause of the outbreaks was due to labour migration. NIMR supervised the Elimination of Lymphatic Filariasis programme in Karnataka.

Chennai (Tamil Nadu)

During the year, studies pertaining to environmental, social and behavioural risk factors related to malaria transmission in Chennai have been initiated. Geographical reconnaissance has been completed in the experimental area and is being carried out in the control area. Assessment of therapeutic efficacy of chloroquine in the treatment of falciparum malaria has been undertaken in Besant Nagar area in Chennai after repeated complaints on malaria among treated patients. Study indicated 38% late treatment failure. Phase III trials of Lambdacyhalothrin 10% CS as indoor residual spray has been initiated and pre-treatment survey undertaken in Hogenekkal and Ootamalai areas in Dharmapuri District, Tamil Nadu. Other activities included technical support to various centres/institutes and collaborative research/scientific work. Health education and training programmes were undertaken as usual. Malaria clinic continued to function catering to the health needs of the public by providing prompt diagnosis and treatment.

Haldwani (Uttaranchal)

Therapeutic efficacy of chloroquine in the treatment of *P. falciparum* malaria was undertaken in Bel Parao PHC and Haldwani and the results revealed 13.6% late clinical failures, 31.8% late parasitological failures and 54.6% adequate clinical and parasitological response. Seroepidemiological studies were undertaken to investigate malaria endemicity in collaboration with NIMR Hqs. A total of 87 blood smears were collected and the study is in progress. Diagnosis and treatment services were provided to the patients reporting with fever to the malaria clinic at the field unit. Technical support was provided by

organising health camps, training courses, group meetings, live demonstrations, etc.

Hardwar (Uttaranchal)

Fraction code MRCHAR/03/05S from plant code MRCHAR/03/05 showed excellent activity against *An. stephensi, Aedes aegypti* and *Culex quinquefasciatus*. Gas chromatography-mass spectrometer analysis showed 80 and 62 peaks, on mass spectrum data base search. Major six components (5–10%) identified were namely (i) 2-Butanone, 4-(2,6,6-trimethyl-2 cyclohexen–1-yl (10.11%); (ii) Patchouli alcohol (8.55%); (iii) Cubenol (5.97%); (iv) Caryophyllene oxide (5.46%); (v) Cadinol (5.23%); and (vi) Aristolene (5.19%). Total contributions of all these six compounds were 40.51%. The work has been filed for patent [Application number 3234 /DEL/2005]. Four plants with codes NBDB 022, NBDB041, NBDB 048 and NBDB 056 have been short-listed for further fractionation and formulation to develop as novel insecticides against mosquitoes. Fraction code MRCHAR/04/04/S possessed good adulticide activity against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. Fraction codes MRCHAR/03/04/1 and MRCHAR/03/04/4 of plant code MRCHAR/03/04 showed good antiplasmodial activity with their IC_{50} values of 0.62 and 1.5 $\mu g\ ml^{-1}$ respectively. Plant code MRCHAR/04/03 from Garhwal region of northwest Himalayas possessed good antipasmodial activity by *in vitro* method. About 246 samples of soil, sediment, water, human blood and human milk collected from Garhwal region were processed for the determination of organochlorine residues.

Bioenvironmental control methods successfully controlled malaria in industrial townships of Bharat Heavy Electricals Limited (BHEL), Hardwar, Indian Drugs and Pharmaceutical Limited (IDPL), Rishikesh and Indian Oil Corporation (IOC), Mathura. Technical support was also provided to other industries. Malaria cases declined at National Thermal Power Corporation (NTPC), Rihand Nagar due to effective control measures. Entomological and parasitological investigations were carried out to know the transmission dynamics of malaria in the villages of Laksar PHC, Hardwar. Studies have indicated the existence of a new *An. fluviatilis* sibling species at Laksar PHC. Efficacy of larvivorous fish *Poecilia reticulata* against mosquito larvae was monitored in a polluted water blocked drain at BHEL, Hardwar, India. *Poecilia reticulata* failed to feed on *Cx. quinquefasciatus* larvae in this drain and preferred to feed upon the other available food present in the polluted water compared to *Culex* larvae.

Jabalpur (Madhya Pradesh)

Study on 'Cerebral malaria associated neurological disorders in India' was carried out at Medical College Hospital, Jabalpur and Maihar Civil Hospital. A total of 199 prospective cases were admitted in the hospital. The case fatality rate was 41%. Base line census of 52 study villages (Pop. 28,789) under the project 'Preparation of a field site for malaria vaccine trial' is completed. HRS book (log book of each village) has been printed as "National resource". About 6800 women of reproductive age were identified to be enrolled. On an average about 80% women were anaemic. Study on 'Transmission dynamics of malaria in tribal areas' showed significantly higher malaria and *P. falciparum* prevalence in Baiga villages of Dindori district as compared to Kanha villages of District Mandla ($\beta = 1.67$ z state 14.4, $p < 0.0001$). The pattern of mosquitoes and vectors were also different. *An. fluviatilis* 'S' was found only in Baiga villages. Clinical trial with α–β Arteether in paediatric age group was carried out in collaboration with Central Drug Research Institute (CDRI), Lucknow and Paediatrics Department of Medical College, Jabalpur. A total of 47 children were enrolled. The results revealed rapid clearance of fever and parasitaemia (24–72 h). No adverse effect was recorded and drug was well tolerated. Studies on migration malaria revealed that prevalence of malaria is still very high in Panna (59% SPR with 83% *Pf*) inspite of intervention measures. A rapid spread of malaria was recorded in all adjoining districts—Katni (SPR 36%), Satna (SPR 42%), Umaria (SPR 57%) and Jabalpur (SPR 31%) where malaria incidence was low. Six malariology training workshops for Medical Officers of various districts of Madhya Pradesh were organised at Jabalpur on the request of Directorate of Health Services, Bhopal.

Nadiad (Gujarat)

Health impact assessment of Sardar Sarovar Water Resources Development Project has made good progress. A large-scale randomised controlled trial of a native fish species (*Aphanius dispar*) has been initiated for malaria control in a semi-arid zone in north Gujarat. New insecticide and larvicides were field evaluated. Technical support was provided to the vector borne disease control programme in Gujarat state for various activities such as planning of antimalaria activities, malaria epidemic investigation/ containment, malaria situation analysis in flood-affected areas, dengue surveillance, preparation of fish hatchery designs, MDA, malaria treatment at clinic, cross-checking of slides, IEC and human resource development.

Panaji (Goa)

Geographical reconnaissance studies were undertaken in Panaji to map the breeding sites of *An. stephensi* and other mosquitoes. The study brought in focus a large variety of habitats supporting breeding of *An. stephensi, Ae. albopictus, Ae. aegypti* and *Cx. quinquefasciatus*. Monitoring of programme implementation was undertaken in North Goa and a detailed report based on findings in epidemiological, entomological questionnaire based surveys was submitted to NVBDCP. Malaria incidence in Panaji after the introduction of sulphadoxine-pyrimethamine (SP) as second line therapy in the treatment of uncomplicated *P. falciparum* malaria was monitored and the results showed desired impact of SP therapy in reducing the malaria incidence. Malaria burden estimation was undertaken in six districts of Chhattisgarh state. Advocacy workshop was conducted at Ranchi and the work was initiated. A survey was conducted in Goa to study the prevalence of dengue vectors and their breeding places and the results confirmed the presence of *Ae. aegypti* and *Ae. albopictus* in Goa and the study will be continued to study the seasonal trends in the population. Evaluation of Parabank, a rapid diagnostic test for the detection of malaria in blood bank samples was undertaken in Goa and the results revealed high sensitivity and specificity of the test kit in the diagnosis of malaria in blood bank samples. Diagnostic and treatment facilities were provided to the patients reporting to malaria clinic at the field unit.

Rourkela (Orissa)

Longitudinal parasitological and entomological studies were carried out in forest and plain area villages that were selected for the development of a site for malaria vaccine trial. On the basis of existing epidemiological data as well as immune status of the study population, 1–5 yr age group are eligible for malaria vaccine trial and as a pre-requisite to the vaccine trial, the methods of data collection and measurements have been standardised. *Bacillus thuringiensis* formulation BtiAS, VCRC B17 strain was evaluated for its efficacy against mosquito larvae and the results revealed that the compound is effective against culicine larvae up to one week and up to two weeks against anopheline larvae. Phase II evaluation of PermaNets, a long-lasting insecticide treated net has been completed and the results revealed high efficacy of these nets in reducing the mosquito densities and high bioefficacy even after 20 washes. Village-scale trial of Olyset nets was initiated. Efficacy of insecticide treated net programme in Mayur Bhanj and Koenjhar districts was undertaken. Detailed report and recommendations on the basis of bioassays and field surveys were submitted to the programme personnel. Assessment of therapeutic response of antimalarials in Koenjhar and Mayur Bhanj districts was undertaken and the results indicated that the pattern of efficacy of chloroquine and sulphadoxine-pyrimethamine in falciparum malaria was highly variable in different parts of the state although with similar epidemiological scenario. Monitoring of programme implementation at PHC level was undertaken in districts with high malaria burden in Orissa. Diagnostic and treatment services were provided at the malaria clinic. Technical support was provided by imparting trainings, organising health camps, meetings, etc.

Shahjahanpur (Uttar Pradesh)

Diagnosis and treatment services were provided to malaria and filariasis patients attending the

clinic. Entomological evaluation was undertaken in Paintapur and Saijana villages in Daudrul PHC where bioenvironmental control strategy was undertaken earlier. Malaria survey was undertaken in Shahjahanpur district to study the prevalence of malaria where large number of fever cases were reported. Monitoring of antimalaria activities were undertaken in two districts of Bihar upon directions of NVBDCP and detailed report regarding measures taken by the health personnel for the control of malaria was submitted. Monitoring of intensified malaria control project under Global Fund was undertaken by the Shahjahanpur field unit in Changlang district of Arunachal Pradesh and Imphal district of Manipur. Group meetings and health camps were organised to sensitise the community in controlling malaria.

Shankargarh (Uttar Pradesh)

Diagnosis and treatment services were provided to patients attending malaria clinic at the field unit. Technical support in epidemic investigation, therapeutic efficacy studies and situational analysis was provided.

Sonapur (Assam)

The major thrust areas of research included, (i) phase III evaluation of long-lasting insecticidal nets (Olyset nets) against *Anopheles minimus* transmitted malaria in Assam; (ii) assessment of therapeutic efficacy of Artemisinin based combination therapy against uncomplicated *P. falciparum* malaria; (iii) clinical trials of α–β Arteether for the treatment of uncomplicated *P. falciparum* malaria in children; and (iv) assessment of State Implementation Capacities under Global Fund supported Intensified Malaria Control Project of NVBDCP. Other activities included technical inputs to strengthen the malaria control activities specific to Northeastern region; these were, health education and capacity building measures, observance of antimalaria month and, mass propagation and distribution of larvivorous fishes (guppy) in town areas. Effective linkages are being developed with the State Health Directorate for control of epidemic malaria, and other institutions for providing evidence based interventions.

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5

Research Supporting Facilities

5.1 Animal house facility

Rabbits, pigeons, domestic fowls, laboratory mice, etc. are being maintained 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 Institute. Laboratory mice were used in screening the antimalarials, host-parasite interaction studies and maintenance of rodent plasmodia at the parasite bank. Experiments on animals were performed with the approval of the Scientific Advisory Committee (SAC) and Institutional Animal Ethics Committee (IAEC) of the Institute.

5.2 Repository of biological material

5.2.1 Mosquito species

Anopheles stephensi

From urban and semi-urban areas

Nehru Place, Delhi
Nathupura, Delhi
Gurgaon, Haryana
Nanak Enclave, Delhi
Hardwar, Uttaranchal

From rural areas

Ladpur, Haryana
Badhdhana, Haryana
Single Line, Punjab
Faridkot, Punjab

Morphological mutants

Red eye (re) – sex-linked recessive
Black larvae (bl) – autosomal semi-dominant
Golden yellow (gy) – autosomal semi-dominant
Creamish white eye (cw) – new mutant
Black larva white eye (blew) – new mutant

Biochemical variant

Bahadurgarh, Haryana (EST-2)

Anopheles culicifacies **Complex**

Species A

Dehra, Uttar Pradesh
Burari, Delhi
Rourkela, Orissa
R6-Rourkela, Orissa
RM-4-Chennai, Tamil Nadu

Species B

Acrocentric Y-chromosome lines
Ladpur, Haryana
Haldwani, Uttaranchal
R39-Rourkela, Orissa

Species C

Submetacentric Y-chromosome lines
Jabalpur, Madhya Pradesh
Rourkela, Orissa

Anopheles fluviatilis **Complex**

Species T

Rourkela, Orissa
Hardwar, Uttaranchal
Haldwani, Uttaranchal

Species U

Hardwar, Uttaranchal

Anopheles sundaicus **Complex**

Andaman & Nicobar Islands

Morphological mutant

Dark green (Larvae) – mutant

Aedes aegypti

Delhi

Culex quinquefasciatus

Delhi

Morphological mutants

Red eye (re)
Scarlet eye (se)

5.2.2. Parasite species

Human Plasmodia

- Non-adapted cryopreserved isolates of *P. falciparum, P. vivax* and *P. malariae*
- Sera/plasma from infected patients

P. falciparum

- Adapted/Characterised isolates
- Different stages of the parasite from culture
- Merozoites (from culture supernatant)
- Ring (by synchronisation)
- Gametocytes (by Hypoxanthine treatment)
- Free parasites for antigen preparation (by Saponin lysis and ultrasonication)

P. vivax

— Sporozoites harvested from artificially fed mosquitoes

Cell Lines

— Hepatoma cell line: Hep G2 A16 used in the *in vitro* cultivation of pre-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 anti-body secreting cells)

Non-human Plasmodia

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

Human malaria parasites in the Parasite Bank

Parasite species	Collection sites (States and districts)	No. of isolates
P. falciparum	**Andhra Pradesh**	
	Visakhapatnam	12
	Assam	
	Sonapur	20
	Tezpur	6
	Chhattisgarh	
	Jagdalpur	14
	Delhi	
	Delhi	191
	Gujarat	
	Anand	4
	Kheda	7
	Haryana	
	Gurgaon	25
	Karnataka	
	Mangalore	14
	Madhya Pradesh	
	Mandla/Jabalpur	14
	Orissa	
	Rayagada	29
	Sundargarh	42
	Rajasthan	
	Alwar	25
	Bharatpur	35
	Jaisalmer	38
	Tamil Nadu	
	Chennai	3
	Ramanathapuram	1
	Uttar Pradesh	
	Baharaich	22
	Gautam Budh Nagar	37
	Ghaziabad	17
	Allahabad	60
	West Bengal	
	Kolkata	18
	Midnapur	1
	Total	635
P. vivax	**Karnataka**	6
	Delhi, Uttar Pradesh and Orissa	53
	Tamil Nadu	3
P. malariae	**Orissa**	5

Details of characterised *P. falciparum* Isolates at the Parasite Bank

Species/Strains of parasite	No. of isolates
Adapted isolates susceptible to chloroquine	54
Adapted isolates resistant to chloroquine	52
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 neuraminidase or trypsin treated erythrocytes	3
Field isolates which can invade both neuraminidase treated and 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
Isolates with isoenzyme profile of GPI, GDH, ADA and LDH markers	22
Isolates with MSP-1, MSP-2 and GLURP markers	40

Non-human malaria parasites available at the Parasite Bank

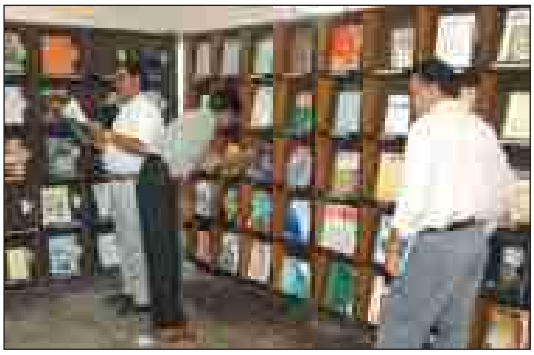
Parasite species	Source	Susceptibility to antimalarials
Simian malaria parasites		
P. cynomolgi bastianelli	NICD, Delhi	Not done
P. knowlesi	NICD, Delhi	Not done
P. fragile	CDRI, Lucknow	Not done
Avian malaria parasites		
P. gallinaceum	NICD, Delhi	Not done
P. relictum	Wild, Delhi	Not done
Rodent malaria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

5.3 Library

The Institute has one of the best libraries in the country in the field of malaria having more than 6735 books, 4083 bound journals, 3573 reprints, 18 video cassettes, 27 audio cassettes, 20 microfilms, 19 theses and 106 national and international reports. About 52 journals (39 Foreign and 13 National) are being subscribed besides 8 journals which are received on complimentary and exchange basis. Two magazines and six newspapers are subscribed. About 201 new books were added during this financial year.

The library renders its services not only to the scientists/research scholars of the Institute but also to various national and international universities and organisations. During the year modernisation process has been expedited and entry into LIBSYS software of about 6308 books and 2000 journals had been completed. Library is also serving its users through DELNET. Other services such as information retrival, citation index, internet facility, inter library loan facility, reprographic services, etc. are also being provided to the users. J Gate and JCCC@ICMR & Proquest Medical database are being provided to NIMR scientists in Delhi and IDVC field units located in different parts of India. In the process of modernisation application of barcode system is under progress.



OBITUARY

Dr. C. Usha Devi (1948–2005)

Dr. Usha Devi joined MRC in 1981 as Assistant Research Officer and served in different capacities for about 25 years. She was Assistant Director when she passed away on 5 October 2005. Her efforts in establishing the Malaria Parasite Bank and also screening of medicinal plants for their antimalarial activity are highly appreciated. She has published more than 35 research papers and her services to this institute shall be remembered always.

6

Inter Institutional Collaboration

Collaborative projects were underatken with the following ICMR/Non-ICMR institutes and medical colleges of the country.

1. 'Development of site for Malaria Vaccine Trial at Sundergarh District, Orissa' in collaboration with International Centre for Genetic Engineering and Biotechnology, New Delhi and State Government of Orissa.

2. 'Raising Antibodies against Synthetic Petides of PfHRP-2 Antigen and LDH using Microsphere Diversity: Development of Diagnostic Reagents' in collaboration with Department of Biotechnology, All India Institute of Medical Sciences, New Delhi.

3. 'Evaluation of Therapeutic Efficacy of Antimalarials' in collaboration with State Governments of Assam, Goa, Gujarat, Madhya Pradesh, Tamil Nadu and Uttaranchal.

4. 'Clinical Drug Trials of Antimalarials' in collaboration with Medical Colleges of Guwahati, Jabalpur, Mangalore Hospital and Tea Estate, Assam.

5. 'Cerebral Malaria Associated Neurological Disorders in Central India' in collaboration with Medical College, Jabalpur, More House School of Medicine and Centre for Disease Control and Prevention, Atlanta, USA.

6. 'Primary Screening of Medicinal Plants from Northeastern States of India for their Antimalarial Activity' in collaboration with Defence Research and Development Organization (DRDO), Tezpur, Assam.

7. 'Screening of Chloroquine Sensitivity Status of *P. falciparum* Parasites from Western Border Areas of India' in collaboration with Defence Research and Development Organization, Gwalior.

8. 'Discovering Antimalarials from Marine Organisms' in collaboration with Kerala University, Trivendrum, Kerala.

9. 'Molecular Characterisation of Nitric Oxide Synthase in *An. culicifacies*: Relevance for Refractory Mechanism' in collaboration with Institute for Cytology and Preventive Oncology, Noida.

10. 'Health Impact Assessment of Indira Sagar Dam and Resettlement Colonies in SSP Reservoir Impoundment Areas in Narmada Valley in Madhya Pradesh' in collaboration with National Institute of Virology, Pune, National Institute of Cholera and Enteric Diseases, Kolkata and Narmada Valley Corporation.

11. 'Prospecting for Botanical Pesticides: Screening of Plant Extracts for Insecticidal and Repellent Activity against *An. stephensi*' in collaboration with Department of Biotechnology, New Delhi.

12. 'Characterisation of *P. falciparum* Strains Prevalent in Northeastern States' in collaboration with Regional Medical Research Centre (RMRC), Dibrugarh, Assam.

13. 'Screening of antimalarial activity of synthetic compounds in *P. falciparum* culture lines' in collaboration with Deptt. of Chemistry, University of Delhi and Indian Institute of Chemical Technology, Hyderabad.

14. 'Larvicidal Activity of Extracts of different parts of a tissue cultured plant (code VA-1 of family Astereaceae)' in collaboration with University of Delhi, Delhi.

15. Situation Analysis of Malaria in Car Nicobar after Tsunami Attack using Ground Surveys and Remote Sensing with Immediate Remedial Measures' in collaboration with GB Pant Hospital, Port Blair, Andaman & Nicobar Islands.

7

Research Papers Published

1. Adak T, Singh OP, Das MK, Wattal S, Nanda N. Comparative susceptibility of three important malaria vectors *Anopheles stephensi*, *Anopheles fluviatilis* and *Anopheles sundaicus* to *Plasmodium vivax*. *J Parasitol* 2005; *91:* 79–82.

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for the treatment of *Plasmodium vivax* malaria in India. *Am J Trop Med Hyg* 2005; *73:* 1108–11.

15. Dunne MW, Singh N, Shukla MM, Valecha N, Bhattacharyya PC, Dev V, Patel K, Mohapatra MK, Lakhani J, Benner R, Lele C, Patki K. A multicentric study of azithromycin, alone and in combination with chloroquine, for the treatment of acute, uncomplicated *Plasmodium falciparum* malaria in India. *J Infect Dis* 2005; *191:* 1582–8.

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8

Other Activities

8.1 Information, education and communication (IEC)

National Science Day celebration

A lecture-cum-interactive discussion by an expert Dr. Mathura Prasad, General Manager (Security), National Thermal Power Corporation, New Delhi, was organised on 28 February 2006 on the occasion of National Science Day. The topic of the speech was "Environment and its effects on Human Health". This year's theme of NSD was "Nurture Nature for the future". All NIMR scientists, officers and research fellows attended the lecture and took active part in interaction and discussion.

Exhibition

An exhibition consisting of 20 panels depicting the profile and major research activities of NIMR was

Dr. M.A. Ansari joined as Director, RMRCT, Jabalpur

Dr. M.A. Ansari, Dy. Director (SG), joined as Director of RMRCT, Jabalpur on 22nd February 2006. He joined Malaria Research Centre (now National Institute of Malaria Research) in 1977 as Senior Research Officer and served in various positions. His main research interests include Vector Biology and Control, particularly development of new vector control tools. He also served as Editor of *Journal of Vector Borne Diseases* and *Malaria Patrika* (Hindi). He has published more than 125 scientific research articles in various national and international journals.

displayed at the venue of International Conference on Malaria held during November 2005. This conference was organised by the National Institute of Malaria Research and attended by various eminent scientists from India and abroad.

Video documentary

A video documentary on the evaluation of Olyset nets manufactured by Sumitomo Chemicals was completed. The video shooting was undertaken in villages of Dadri PHC of District Ghaziabad as well as in the laboratory. Interviews of villagers and scientists were recorded. The editing was completed with incorporation of animation and special effects.

8.2 Publications

Journal of Vector Borne Diseases

The Institute has been publishing an English quarterly, *Journal of Vector Borne Diseases*. The journal publishes original research articles, research communications, review articles on various aspects of different vector borne diseases. The journal is abstracted by major abstracting agencies and is being uploaded in the web for easy access to the scientists working in the field of vector borne diseases. PDF version of all articles published in the journal can be downloaded free of cost from www.mrcindia.org/journal.

Malaria Patrika

Malaria Patrika, a quarterly periodical in Hindi language is published by the Institute to create awareness on malaria in the community. Various activities relating to the Institute, news and latest developments in the field of malaria research are being covered in this periodical apart from scientific articles. The *Patrika* is being applauded by the community.

Sandarbh Pustika

A reference booklet in Hindi titled "*Rajbasha karyanvayan sambandhi sandarbh pustika*" was published in 2005 to promote Hindi as an official language and to implement official language rules. This booklet contains highlights of official language rules, vocabulary related to administrative terms and selected notings that are regularly used in day- to-day office work.

Proceedings of the Drug Policy Workshop

A document on the proceedings of the Drug Policy Workshop was published on the guidelines for use of antimalarials and in the treatment of malaria. Detailed therapy of *P. vivax* malaria, uncomplicated and complicated malaria, severe malaria, *P. falciparum* malaria, etc was presented along with the annexures of Drug Policy of National Vector Borne Disease Control Programme, Govt. of India.

Website www.mrcindia.org

The Institute's website contains information on research activities being carried out by NIMR, publications of the Institute, research activities of the field units of NIMR, audio-visual unit activities and profiles of scientists, etc. The documents and books published by the Institute are available in this website. A separate webpage is being maintained for Institutes's English periodical, the *Journal of Vector Borne Diseases*. The website contains information on guidelines for contributors, subscribers and contact information of editorial office, etc.

8.3 Conferences/Workshops/Seminars attended and papers presented/Lectures delivered

International Conference on Malaria, 4–6 November 2005, New Delhi, India

All the scientists of the Institute and IDVC Field Units attended the conference and presented papers.

Prof. A.P. Dash

1. Chaired the scientific session on "Infection in immuno-compromised hosts & host response' at the Indo-German workshop on 'Recent advances in global research in infectious diseases" at Braunschweig, Germany from 15–19 June 2005.
2. 'Drug policy workshop' from 29–30 October 2005 at NIMR, Delhi.
3. Conference on 'Multilateral initiative on malaria' at Yaounde, Camaroon from 12 to 18 November 2005.
4. International conference on 'Vivax malaria research: 2005 and beyond' in the Institute of Genomic Research (TIGR), Rockville, Maryland and New York University, USA from 5–7 and 9–10 December 2005.
5. 'III southeast Asia and western pacific bi-regional TEPHINET' conference at Chennai on 8 January 2006.
6. National symposium on 'Recent trends in malaria studies' at Pune on 20 January 2006 and delivered the key note address.
7. 'Urban malaria' workshop at Ajmer on 23 January 2006.
8. International conference on 'Urban Health Initiatives' at Surat from 8–9 February 2006.
9. Workshop on 'Drinking water & community health standard' at Indian National Science Academy on 10 February 2006.
10. VI Joint annual conference of Indian Society of Malaria and other Communicable Diseases (ISMOCD) and Indian Association of Epidemiologists (IAE) at Agra from 11–13 February 2006 and delivered invited lecture on 'Malaria: Lessions Learnt'.
11. 'Trainer's training workshop' at Bangalore from 14–15 February 2006.
12. Delivered Dr. C.P. Alexander Memorial Lecture on 'Malaria: are we loosing the battle?' at the Deptt. of Zoology, University of Delhi, Delhi on 10 March 2006.

Dr. T. Adak

1. National seminar on 'Integrated pest management' organised by Ministry of Agriculture, Deptt. of Agriculture and Cooperation at New Delhi on 26 July 2005.

Dr. R.C. Dhiman

1. Panel discussion as an invitee in DFID country and regional consultative workshop on 'Climate change and development' at TERI, New Delhi on 5 April 2005.

Dr. Hema Joshi

1. 'Medicine and health in the tropics', Marseille, France from 11–15 September 2005. Presented a paper on "*Plasmodium falciparum*: genetic polymorphism and identification of recrudescent infections in field isolates of India".
2. International conference on 'Vivax malaria research: 2005 and beyond' the Institute of Genomic Research (TIGR), Rockville, Maryland from 9–10 December 2005. Presented papers on "*P. vivax*: genetic complexity among field isolates in India" and "Highly polymorphic marker in Indian field isolates".
3. Tefenoquine *P. vivax* Advisory Board Meeting, Bangkok, Thailand from 13–14 February 2006. Presented a paper on "Vivax malaria in India".
4. USAID project workshop on "Therapeutic efficacy of antimalarials", at Sonapur (Assam).

Dr. P.K. Mittal

1. National workshop on 'Application of pesticide formulation' organised by the Institute of Pesticide Formulation Technology, Gurgaon and held at Scope Convention, Delhi from 10–13 May 2005.
2. Brain storming workshop on 'Technology for rural women' organised by the Department of Rural Development and Technology, IIT, New Delhi on 12 May 2005. Delivered a lecture on "Indigenous methods for vector control".

Dr. B.N. Nagpal

1. Conference of American Geophysical Union (AGU), New Orleans, Louisiana, USA from 23–27 May 2005. Presented a paper on "An integrated atmospheric and hydrological-based malaria epidemic alert system".
2. International symposium on 'Health GIS 2005' held at Bangkok, Thailand from 1–2 December 2005. Presented a paper on "Cost-effective control of malaria using GIS in forest-fringe areas in India".
3. "Urban malaria – Survey report of Ajmer" paper presented in a workshop on 'Urban malaria' under USAID project at Ajmer on 24 January 2006.
4. International conference on 'Urban Health Initiatives' organised at Surat from 9–11 February 2006. Presented a paper on "GIS-based malaria information management system for urban malaria".
5. "Urban malaria – Survey report of Delhi" paper presented in a workshop on "Urban malaria" under USAID project: (i) for Shahdara South and Shahdara North zones at NIMR, Delhi on 6 February 2006; (ii) for Civil Lines, Rohini and Narela zones at IPP-VIII Office, C.L. Zone on 21 February 2006; (iii) for Karol Bagh, West Delhi and Najafgarh zones at West Zone meeting hall on 24 February 2006; (iv) for City and S.P. zones at Asaf Ali Road, New Delhi on 25 February 2006; and (v) for South and Central zones at South Zone meeting hall on 6 March 2006.
6. Workshop on 'Environment and its effects on health' organised by NIMR on 28 February 2006.
7. Workshop to review the situation of vector borne diseases organised by MCD held at India Habitat Centre, New Delhi on 2 March 2006.
8. Workshop on 'National consultation meeting on malaria burden in India' at National Agriculture Science Complex, New Delhi from 29–30 March 2006.

Dr. Nutan Nanda

1. Conference on 'Vector biology and control' held at New Delhi from 4–6 November 2005.

Dr. K. Raghavendra

1 ICMR-MIHR symposium on 'Living with TRIPS–Innovation of new health technologies for the poor' held at New Delhi from 5–7 December 2005.
2. 'International conference on Copepoda' held at Hamananet, Tunisia in July 2005. Presented a paper on "Effect of insecticides (temephos and fenthion) on larvivorous efficacy of *Mesocyclops thermocoides* (Copepoda: Cyclopoidae) under experimental conditions".
3. "Perspectives on the global challenge to malaria research: Indian response. Potential synergies with South Africa" in the workshop on 'Malaria initiative for Africa' organised by CSIR and DST, South Africa held at Pretoria, South Africa from 4–7 September 2005.
4. National conference on 'Plant tissue culture and biotechnology' at Central Institute of Medicinal and Aromatic Plants, Lucknow from 8–9 December 2005. Presented a paper on "Micro propagation of traditional medicinal plant Akar kara (*Sphillanthus acmella* L.) and evaluation of its larvicidal activity against malaria vectors *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say".

5. 'VI Joint annual conference of Indian Society of Malaria and other Communicable Diseases (ISMOCD) and Indian Association of Epidemiologists (IAE), at Agra, Uttar Pradesh from 11–13 February 2006. Presented papers on (i) "Current scenario of insecticide resistance among arthropod vectors of medical importance and genetically modified mosquitoes and prospects for malaria control"; (ii) "Effect of nutrition on efficacy of *Lagenidium giganteum* couch against mosquito larvae"; and (iii) "Larvicidal activity of crude aqueous extracts of *Xanthium stramarium* (Family: Astereaceae)".
6. National consultative workshop on 'National Vector Borne Disease Control Programme', organised by NVBDCP at New Delhi on 16 March 2006 and presented the status report and recommendations on the control of Japanese encephalitis.
7. III International malaria research conference jointly organised by the Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health at Baltimore, Maryland, USA from 20–21 March 2006 and Dr. S.S. Mohanty presented a poster entitled, "Efficacy of extracellular metabolites of *Metarhizium anisopliae* against mosquito larvae".

Dr. A.M. Reetha

1. Drug policy workshop at NIMR, Delhi from 26–29 October 2005.

Mrs. Rekha Saxena

1. 'Conference of American Geophysical Union (AGU)', New Orleans, Louisiana, USA from 23–27 May 2005 and presented a paper on "An integrated atmospheric and hydrological-based malaria epidemic alert system".
2. International symposium on 'Health GIS 2005', held at Bangkok, Thailand from 1–2 December 2005 and presented a paper on "Cost-effective control of malaria using GIS in forest-fringe areas in India".

Dr. Aruna Srivastava

1. 'Conference of American Geophysical Union (AGU)', New Orleans, Louisiana, USA from 23–27 May 2005 and presented a paper on "An integrated atmospheric and hydrological-based malaria epidemic alert system".
2. International symposium on 'Health GIS 2005' held at Bangkok, Thailand from 1–2 December 2005 and presented a paper on "Cost-effective control of malaria using GIS in forest-fringe areas in India".
3. "GIS for urban malaria" paper presented in a workshop on 'Urban malaria' under USAID project, held at Ajmer on 24 January 2006.
4. International conference on 'Urban health initiatives' organised at Surat from 9–11 February 2006 and presented a paper on "GIS-based malaria information management system for urban malaria".
5. "GIS for urban malaria" paper presented in a workshop on 'Urban malaria' under USAID project: (i) for Shahdara South and Shahdara North zones at NIMR, Delhi on 6 February 2006; (ii) for Civil Lines, Rohini and Narela zones at IPP-VIII Office, C.L. Zone on 21 February 2006; (iii) for Karol Bagh, West Delhi and Najafgarh zones at West Zone meeting hall on 24 February 2006; (iv) for City and S.P. zones at Asaf Ali Road, New Delhi on 25 February 2006; and (v) for South and Central zones at South Zone meeting hall on 6 March 2006.
6. Workshop on 'National consultation meeting on malaria burden in India' at National Agriculture Science Complex, New Delhi from 29–30 March 2006.

Dr. Neena Valecha

1. 'Joint ICMR/DSPRUD symposium on anti-microbial resistance and antibiotic use' held on 13 April 2005 at ICMR, New Delhi and delivered a lecture on "Surveillance of antimicrobial resistance for *Plasmodium*".

2. National consultation meeting on 'Drug resistance in malaria, TB and HIV/AIDS' at Mumbai, India from 19–21 September 2005 and delivered a lecture on "Monitoring of drug resistance".
3. National conference on 'Infectious diseases' at Sir Ganga Ram Hospital, New Delhi on 18 December 2005 and delivered a lecture on "Treatment of malaria".
4. Workshop on the 'Epidemiology of urban malaria in mega, medium and small cities of India' at Ajmer on 24 January 2006 and delivered a lecture on "Treatment of malaria".
5. International conference on 'Urban health initiatives' at Surat Municipal Corporation, Health & Family Welfare Department, Govt. of Gujarat on 9 February 2006 and delivered a lecture on "Drug resistance".
6. Workshop on the "Epidemiology of urban malaria in mega, medium and small cities of India" at Delhi on 21, 24, 25 February, and 6 March 2006 and delivered a guest lecture on "Treatment of malaria".
7. Workshop on 'Malaria initiative for Africa' organised by CSIR and DST, South Africa held at Pretoria, South Africa from 4–7 September 2005.
8. International conference on 'Vivax malaria research: 2005 and beyond' at TIGR, Maryland, USA from 9–10 December 2005.

8.4 Important meetings attended

Prof. A.P. Dash

1. Expert Core Group meeting at NVBDCP, Delhi on 14 April 2005.
2. Scientific Advisory Committee meeting of Desert Medical Research Centre, Jodhpur from 15–16 April 2005.
3. Launching of Antimalaria month campaign and logo for National Vector Borne Disease Control Programme and release of Operational guide on antimalaria month campaign at Vigyan Bhawan, New Delhi on 5 May 2005.
4. Japanese encephalitis and dengue meeting at Pune on 13 May 2005.
5. Japanese encephalitis meeting at NVBDCP, Delhi on 1 June 2005.
6. Technical Advisory Committee meeting at Delhi on 7 July 2005.
7. Kala-azar meeting at India Habitat Centre, New Delhi on 12 July 2005.
8. Task Force meeting on Lymphatic filariasis at ICMR, New Delhi on 19 July 2005.
9. Epidemiology week celebrations at Chennai from 24–28 July 2005.
10. Tribal malaria meeting at ICMR, New Delhi on 6 September 2005.
11. Global Forum for health research at Mumbai from 12–13 September 2005.
12. National consultation meeting on 'Drug resistance in malaria, tuberculosis, HIV/AIDS, at Mumbai from 19–20 September 2005.
13. Scientific Advisory Committee meeting of the Centre for Research in Medical Entomology at Madurai on 30 September 2005.
14. Library Expert Committee meeting at ICMR, New Delhi on 10 October 2005.
15. Use of larvicides meeting at NVBDCP, Delhi on 15 October 2005.
16. Indo-French collaborations meeting at ICMR, New Delhi on 24 October 2005.
17. Task Force meeting at Vector Control Research Centre, Pondicherry on 9 November 2005.
18. Expert Committee meeting at UGC, New Delhi from 16–17 January 2006.
19. Joint working group meeting at ICMR, New Delhi on 18 January 2006.
20. Prevention of biomedical risks meeting at French Institute, Pondicherry from 2–4 March 2006.
21. Indo-German meeting on microbiology, immunology & cell biology: perspectives for Indo-German Research Cooperation" at ICGEB, New Delhi on 12 March 2006.

22. Training session meeting on Integrated Vector Borne Disease Control Programme at NICD, Delhi on 27 March 2006.
23. Launching of Satellite Linkage Integrated Diseases Surveillance meeting at NICD, Delhi on 29 March 2006.

Dr. R.C. Dhiman

1. NATCOM consultative meeting (Invited) on 'Development of a framework for vulnerability assessment and adaptation for water resources, agriculture, coastal zones and human health' held at IIT, New Delhi on 22 July 2005.
2. Indo-UK meeting of ERM at New Delhi on 8 September 2005.
3. Health and environment meeting at ICMR (HQ), New Delhi in December 2005.

Dr. Hema Joshi

1. Technical committee meeting on "Drug resistant malaria" organised by NVBDCP, Delhi.
2. Meeting related to Genome project with Prof. Dyann Wirth, Boston University, USA on "Haplotype map of *P. falciparum*" at ICMR, New Delhi on 4 October 2005.
3. Indo-German research cooperation meeting from 11–12 March 2006.

Dr. P.K. Mittal

1. Review meeting of Kala-azar control programme for elimination by 2010, organised by the National Vector Borne Disease Control Programme at India Habitat Centre, New Delhi on 12 July 2005.

Dr. B.N. Nagpal

1. Meetings on the progress of work done by NIMR, Delhi for ISP, OSP and SSP organised by NVDA, Bhopal on 12 April and 16 August 2005 and on Control of malaria for ISP, OSP and SSP on 9 May 2005.
2. GIS in malaria control and morphotaxonomy meeting organised at DRDE, Gwalior on 4 May 2005.
3. Kala-azar control programme meeting organised by NVBCDP at Delhi on 12 July 2005.
4. National task force for implementation of National Anti Malaria Management Information System (NAMMIS) meeting organised by NVBDCP at Delhi on 5 August and 9 November 2005.
5. Meeting to review the dengue situation organised by the Director, MCD at Town Hall, Delhi on 24 August 2005.
6. Expert group of dengue organised by the Directorate of Health Services, Delhi under the chairmanship of Principal Secretary (Health) at Delhi Secretariat on 16 September 2005.
7. Assessment of malaria treatment practices in public and private health sectors, under USAID project organised by NIMR at NICD, Delhi under the chairmanship of Addl. Director General & Director, NICD on 27 December 2005 and 22 March 2006.
8. Meeting to review action plan and action taken by the states for prevention and control of dengue organised by NVBDCP, Delhi at Nirman Bhavan, New Delhi on 10 March 2006.
9. Half yearly review meeting of the NBDB (DBT) funded project on Prospecting for botanical pesticide held at EID Parry R&D Centre, Bangalore on 20 July 2005.

Dr. Aruna Srivastava

1. Meeting with the Director, DRDE, Gwalior on 'GIS in malaria control in Cantonment area', at DRDE, Gwalior on 4 May 2005 and presented a paper on "GIS for decision support in malaria control".
2. 'Control of malaria for ISP, OSP and SSP' organised by the State Health Department under the chairmanship of Advisor (Health) at NVDA, Bhopal on 9 May 2005.

3. ICMR Forum for epidemiology organised by the National Institute of Epidemiology, Chennai on 25 July 2005.
4. Review meeting for ISP, OSP and SSP organised by NVDA, Bhopal under the chairmanship of VC on 16 August 2005.
5. National task force meeting for implementation of National Anti Malaria Management Information System (NAMMIS) at NVBDCP, Delhi on 9 November 2005.
6. 'Assessment of malaria treatment practices in public and private health sectors' under USAID project organised by NIMR at NICD, Delhi under the chairmanship of Addl. Director General & Director, NICD on 27 December 2005 and 22 March 2006.
7. For improvment of the Website of ICMR, organised by ICMR, New Delhi on 12 January 2006.

Dr. Neena Valecha

1. 'Development of a multi-country proposal for malaria' held at GFATM, Bangkok, Thailand from 25–29 April 2005.
2. Member Advisory Board for "MMV/Ranbaxy Synthetic Peroxide (RBx-11160/OZ 277) Project Development Advisory Board Meeting", Geneva from 17–19 May 2005.
3. APW with WHO for preparation of document on Drug resistance of malaria in SEARO.
4. National consultation on drug resistance in malaria TB and HIV/AIDS at Mumbai, India from 19 –21 September 2005.
5. 'Phase III clinical trials for antimalarial drugs: study design issues' at Bethesda, Maryland, USA on 8 December 2005.
6. Launching of Antimalaria month campaign and Logo for National Vector Borne Disease Control Programme and release of Operational guide on antimalaria month campaign at Vigyan Bhawan, New Delhi on 5 May 2005.
7. 'Technical specification of rapid diagnostic kits for malaria' at NVBDCP, Delhi on 22 June 2005.
8. Technical Advisory Committee (TAC) on malaria at DGHS, Nirman Bhawan, New Delhi on 7 July 2005.
9. 'The collaboration of drugs for neglected diseases initiatives (DnDi) and ICMR' at ICMR, New Delhi on 5 August 2005.
10. 'Ongoing drug sensitivity trials undertaken jointly by MRC, ROH&FW' at Chennai from 16–17 August 2005.
11. 'Quality assurance on laboratory diagnosis of malaria' at Directorate of NVBDCP, Delhi on 24 August 2005.
12. 'A phase II, multi-centre, double-bind, dose ranging, safety and efficacy study of RBx 11160 in patients with uncomplicated *Plasmodium falciparum* malaria' at Rourkela, Orissa from 28–30 August 2005.
13. Global Forum for health research at Mumbai from 12–13 September 2005.
14. Orientation meeting on 'Assessment of therapeutic efficacy of antimalarial drugs against uncomplicated *P. falciparum* malaria' at Guwahati, Assam on 19 October 2005.
15. Independent ethics committee meeting at Essex Farm, New Delhi on 11 December 2005.
16. 'Medical development congress' meeting at New Delhi on 23 January 2006 .
17. Interactive session on vector borne disease and presentation on 'Antimalarial drug resistance studies in India' at ICMR, New Delhi on 30–31 January 2006.
18. Meeting to discuss 'NIMR clinical trials activities, response to protocol for Euartekin trials' at Delhi on 5 February 2006.
19. Advisory Board meeting for 'Synthetic peroxide (RRx 11160/OZ 277) PDT meeting' organised by MMV at Ranbaxy Laboratories, Gurgaon from 6–7 February 2006.
20. 'How to enhance Indo-German research cooperation?', at the Seminar Room, Indo-German Chamber of Commerce (IGCC), German House, New Delhi on 11 March 2006.

21. Indo-German meeting on Microbiology, immunology & cell biology: perspectives for Indo-German research cooperation at ICGEB, New Delhi on 12 March 2006.

8.5 Conferences/Workshops/Meetings organised

Conference

International Conference on Malaria

Malaria Research Centre (now, National Institute of Malaria Research) organised an International Conference on Malaria which was held at New Delhi from 4–6 November 2005. The conference was organised to mark the 125th anniversary of **Laveran's** discovery of malaria parasite in human blood on 8 November 1880. For this work he was awarded the 1907 Nobel Prize for Physiology/Medicine. The theme of the conference was 'Laveran to Genomics'. The conference was held at National Agriculture Science Complex (NASC), New Delhi and inaugurated by Mrs Panabaka Lakshmi, Honourable Minis-

ter of State for Health and Family Welfare, Govt. of India. Dr. Samlee Plianbangchang, Regional Director, World Health Organization, South-East Asia Regional Office, New Delhi, addressed the gathering. The conference was attended by over 500 national and international delegates and featured plenary lectures, invited lectures and free oral and poster presentations on various aspects of malaria.

Workshops

1. 'Identification and *in vitro* cultivation of malaria parasites, screening of antimalarials, establishment of malaria parasite bank' for 17 Ph.D. students and academicians in Sudan during 28 May to 12 June 2005.
2. 'Drug policy workshop' on 30 August 2005 at Rourkela.
3. Five workshops on 'Urban malaria' under USAID project for DHOs, entomologists, AMOs, GPs, pathologists and NGOs etc. of 12 zones of Delhi (MCD) during February and March 2006 at NIMR, Delhi.
4. 'Urban malaria' under USAID project for DHOs, entomologists, AMOs, GPs, pathologists, and NGOs etc. of Ajmer City on 24 January 2006 at Ajmer, Rajasthan.

Meetings

1. Drug policy for malaria at NIMR, Delhi from 26–29 October 2005.
2. Assessment of malaria treatment practices in public and private health sectors" at NIMR, Delhi on 27 December 2005.
3. National consultation meeting on "Malaria burden in India" held at National Agriculture Science Complex, New Delhi from 29–30 March 2006.
4. Assessment of malaria treatment practices in public and private health sectors at NIMR, Delhi on 22 March 2006.
5. Assessment of therapeutic efficacy of antimalarial drugs against uncomplicated *P. falciparum* malaria at Ranchi, Jharkhand, from 23–25 March 2006.

8.6 Trainings imparted

Dr. Hema Joshi

Imparted training and supervised the work of the following students:

1. Mr. Pankaj Sharma, Jiwaji University, Gwalior, Madhya Pradesh.
2. Ms. Shabana Parveen, Jiwaji University, Gwalior, Madhya Pradesh.
3. Mr. Rajneesh Sharma, Chaudhary Charan Singh University, Meerut, Uttar Pradesh.
4. Ms. Nidhi Ralli, CET-IILMAHL (U.P. Technical University), Noida, Uttar Pradesh.
5. Mr. Shameemul Haque, Jamia Milia Islamia, Delhi

Dr. P.K. Mittal

1. Participated as faculty member and delivered lectures on Biological control of mosquitoes to the participants of training course on malariology organised by NICD from 14 February to 11 March 2005 at NICD, Delhi.

Dr. B.N. Nagpal

1. A five-week training imparted to two B. Tech students of Indian Institute of Information Technology, Allahabad on the 'Development of software of inventory control' from 23 May to 30 June 2005.
2. One month training imparted to a student of Universal Public School, Preet Vihar, Delhi to develop 'Software on the identification of mosquitoes' in the month of June 2005.
3. Training imparted to two UNC's undergraduate students—Ms. Allison Booth and Ms. Erica MacKenzie on 'GIS and mosquito identification' at MRC, Delhi on 15 June 2005.
4. A five-day training imparted to two scientists (Dr. R.K. Hazra, SRO & Dr. S.K. Parida, TO) of RMRC, Bhubaneswar on 'GIS and RS in studying the malaria vectors – Biodiversity' in the month of July 2005.
5. Training imparted to 20 participants of Professional Development Course in Management, Public Health & Health Sector Reforms for District Level Senior Medical Officers from State Institute of Health & Family Welfare, Punjab, at NIMR, Madhuban, Delhi on 17 November 2005.
6. Two weeks training imparted to Mr. Drirh, TO, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow on 'Mosquito species identification' in the month of February 2006.

Dr. Nutan Nanda

1. Training in cytotaxonomic techniques for iden-

tification of the members of *An. culicifacies* and *An. fluviatilis* complexes provided to Mr. K. Vaidyanathan and Mr. A. Agatheeswarani, technical staff from Vector Control Research Centre for two weeks in May 2005.

2. Training in cytological identification of *An. culicifacies* and *An. fluviatilis* sibling species and mosquito blood meal source analysis using counter current immunoelectrophoresis technique provided to Mr. Amitav Mohanty, Research Fellow from the Institute of Life Sciences, Bhubaneswar in May–June 2005.
3. Trained in processing of mosquitoes for cytotaxonomic and bionomics studies and cytological identification of sibling species to Dr. R.K. Hazra, Senior Research Officer from the Regional Medical Research Centre, Bhubaneswar in July 2005.

Dr. C.R. Pillai

1. Dr. N.C. Gupta, Technical Officer, IDVC field unit Hardwar was imparted training on '*In vitro* cultivation of malaria parasite *P. falciparum* and preparation of plant extracts and their testing in antimalarials' from 9–14 May 2005.
2. Dr. Dipak Chetia, Deptt. of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam was imparted training on '*In vitro* cultivation of malaria parasite *P. falciparum* and preparation of plant extracts and their testing in antimalarials' from 14–16 July 2005.
3. Dr. Swati Bhattacharyya, P.G. student, Deptt. of Pharmacology, Mahatma Gandhi Institute of Medical Sciences, Sewagram, Maharastra, was imparted training on 'Identification and *in vitro* cultivation of malaria parasite *P. falciparum*' from 21–25 November 2005.
4. Mr. Pankaj Gupta, M. Pharma, Guru Jambheshwar University, Hisar, Haryana, was imparted training on identification and *in vitro* cultivation of malaria parasite *P. falciparum* from 28 November to 2 December 2005.
5. Dr. C. Rajendran, Scientist 'B' from DRL (DRDO), Tezpur, Assam was imparted training on 'Identification and *in vitro* cultivation of malaria parasite *P. falciparum*' from 1–3 March 2006.
6. Mr. Santosh Anand Sripada, Deptt. of Animal Sciences, School of Life Sciences, University of Hyderabad was imparted training on 'Identification and *in vitro* cultivation of malaria parasite *P. falciparum*' from 7–17 March 2006.
7. Mr. Sanket Soni and Mr. Yogesh Biradar from Department of Pharmacognosy and Phytochemistry, B.V. Patel Pharmaceutical Education and Research Development Centre, Ahmedabad, were imparted training on 'Identification and *in vitro* cultivation of malaria parasite *P. falciparum*' from 23 March to 7 April 2006.
8. Miss Ritu Goyal and Miss Poonam from Department of Chemistry, University of Delhi, Delhi, were imparted training on 'Identification and *in vitro* cultivation of malaria parasite *P. falciparum*' from 27 March to 7 April 2006.

Dr. K. Raghavendra

1. Imparted one week training to Dr. Karamveer Singh, DMRC, Jodhpur on 'Microplate assay in determining biochemical resistance mechanism in mosquitoes' from 12–16 December 2005.
2. Provided guidance to accomplish dissertation work (6 months) for partial fulfilment of the M.Sc. degree of the following students:
   - Ms. Pooja Chaturvedi (M.Sc., II yr.) Rani Durgavati University, Jabalpur, Madhya Pradesh on 'Isolation of new strains of bacteria for mosquito larvicidal activity'.
   - Ms. Usha Rai (M.Sc., II yr.), Jiwaji University, Gwalior, Madhya Pradesh on 'Isolation of new strains of fungus for mosquito larvicidal activity and molecular methods for the identification of sibling species'.
   - Ms. Archana Chauhan (M.Sc., II yr.), Jiwaji University, Gwallior, Madhya Pradesh on 'Biochemical mechanisms of resistance and molecular methods for the identification of sibling species'.

Dr. A.M. Reetha

1. Training to students of University of North Carolina, United States on 13 June 2005.
2. Training to Dip. NB Students (Microbiology) from Sir Ganga Ram Hospital on 21 June 2005.
3. Training to WHO fellows from DPR Korea on 7 October 2005.
4. Training workshop for IMA doctors on 'Assessment of malaria treatment practices in public & private health sectors' at Delhi on 22 March 2006.
5. Training workshop for the Medical Officers of Armed forces at Army Base Hospital, Lucknow from 23–25 March 2006. (Resource person – Treatment [uncomplicated and complicated cases] and management of severe and complicated malaria).
6. Training course on 'Integrated vector borne diseases control programme at NICD, Delhi—Panel discussions on: (i) Chemotherapy of malaria in drug policy; and (ii) New diagnostic tools (Rapid diagnostic methods) on 31 March 2006.

Mrs. Rekha Saxena

1. A 19-day training on 'Application of geographical information system (GIS) to study malaria scenario in Jodhpur district, Rajasthan', to Dr. D. Sukumaran, Scientist-D from Defence Research & Development Establishment, Gwalior from 5–23 September 2005.
2. A five-day GIS training to Dr. R.K. Hazra, SRO, Regional Medical Research Centre, Bhubaneswar during July 2005.
3. Training on 'GIS in malaria control, computer-based identification of Indian anophelines & fauna system' to Mr. Manas Sarkar, JRF and Mr. Vipul Ravha, Senior Technical Assistant, DRL, Tejpur (Assam) on 23 December 2005.
4. Training on 'GIS in malaria control, computer-based identification of Indian anophelines & fauna system', to District-level Senior Medical Officers from State Institute of Health & Family Welfare, Punjab during 'Professional Development Course in Management, Public Health & Health Sector Reforms', from 19 September to 26 November 2005.
5. Training to 20 students from Universal Public School, Preet Vihar, Delhi on 11 May 2005. Delivered lectures and made students work on GIS modules and identification of Indian anophelines in celebration of Technology Day.
6. Training on 'GIS in malaria control, computer-based identification of Indian anophelines & fauna system', to two UNC's undergraduate students Ms. Allison Booth and Ms. Erica MacKenzie on 15 June 2005.

Dr. Aruna Srivastava

1. A five-week training to two B. Tech students of Indian Institute of Information Technology, Allahabad on the development of 'Software of inventory control' from 23 May to 30 June 2005.
2. One month training to a student of Universal Public School, Preet Vihar, Delhi to develop software on 'Identification of mosquitoes' in the month of June 2005.
3. Training to two UNC's undergraduate students—Ms. Allison Booth and Ms. Erica MacKenzie on 'GIS and mosquito identification' at MRC, Delhi on 15 June 2005.
4. A five-day training to two scientists (Dr. R.K. Hazra, SRO and Dr. S.K. Parida, TO) of RMRC, Bhubaneswar on 'GIS and RS in studying the malaria vectors– Biodiversity' in the month of July 2005.
5. Training to 20 participants of Professional Development Course in Management, Public Health & Health Sector Reforms for District Level Senior Medical Officers from State Institute of Health & Family Welfare, Punjab at NIMR Madhuban, Delhi on 17 November 2005.
6. A two-week training to Mr. Drirh, TO, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow on 'Mosquito species identification' in the month of February 2006.

Dr. Neena Valecha

1. Teaching/Training/Lectures in Malariology Courses.
2. Training to US-Student of University of North Carolina on 13 June 2005.
3. Training to DNB microbiology students from Sir Ganga Ram Hospital on 21 June 2005.
4. Lecture on 'New diagnostic tools' at NICD, Delhi on 10 February 2006.
5. Lecture on epidemiology, treatment and diagnosis of malaria, at "Trainer's training for the faculty of medical colleges on 'Strategy for ELF and control of vector borne diseases', Chandigarh from 13 to 14 February 2006.

8.7 Ph.D. programme

The following students are working for their Ph.D. degrees under the supervision of NIMR scientists.

Name of the supervisor	Name of the student
Prof. A.P. Dash	Mr. S. Mishra Mr. N. Marai Ms. Priyanka Kar
Dr. M.A. Ansari	Mr. U. Sreehari
Dr. T. Adak	Mr. Anil Sharma Ms. A. Mehrunnisa
Dr. V.K. Dua	Mr. N.C. Gupta Mr. A.C. Pandey Mr. V.P. Ojha* Mr. Firoz Alam Mr. Swapnil Roy
Dr. R.C. Dhiman	Mrs. Sharmila Pahwa
Dr. Ashwani Kumar	Mrs. Deeparani Prabhu Mrs. Nandini Korgaonkar Mr. M.B. Kaliwal
Dr. Arun Sharma	Mr. Suprio Ray Miss Neha Chauhan
Dr. P.K. Mittal	Mr. Suresh Yadav
Dr. Hema Joshi	Mr. S.K. Prajapati Ms. Gertrude Kiwanuka Mr. P.K. Mallick
Dr. Sarala K. Subbarao (Former Director)	Mr. O.P. Singh Mr. Dinesh Chandra

8.8 NIMR Building Complex at Dwarka, New Delhi

At present NIMR is functioning from four different places in Delhi and Noida making it difficult to work in effective and coordinated manner. The institute plans to have its own campus on 7 acre plot in Dwarka taken on long-term lease from Delhi Development Authority. This would include Research Block, Animal House, Auditorium, Hostel, Guest House and Staff Quarters.

Construction of the Research Block

After obtaining necessary approvals from Indian Council of Medical Research the notice inviting tenders (NIT) for construction of Research Block was

published in all leading newspapers on 15 July 2005. The Tender Opening Committee scrutinized the technical and financial bids of the tenderers and their recent works were inspected by the Technical Sub-committee. With the approval of the Building Committee of NIMR and ICMR the construction work of the Research Block was awarded to the L-1 bidder M/s. Rajasthan State Road Development and Construction Corporation Limited, Setu Bhawan, Jaipur (A Rajasthan Govt. Undertaking). The letter of acceptance was issued on 6 January 2006 and as per the conditions of the contract an agreement between the Employer (NIMR) and the Contractor (M/s. RSRDCCL) was signed on 2 February 2006.

The foundation stone laying ceremony of the Research Block was held on the auspicious day of "*Basant Panchami*" (2 February 2006). The foundation stone was laid by Prof. N.K. Ganguly, Director General, ICMR, Prof. R.C. Mahajan, Chairman SAC, Dr. S. Pattanayak, Chairman RAC (IDVC), members of RAC/SAC committees of the Institute, scientists and technical experts from ICMR Hqs and other institutes and staff of NIMR were present on this occasion. The Director General, ICMR congratulated the Director and staff of NIMR on this historic event and promised to extend all possible help in construction of NIMR building complex. The Director, NIMR expressed his gratitude to the DG, ICMR, other officials at ICMR Hqs, the members of Building Committees and technical experts from other institutes for providing constant support and help in every possible manner.

The construction work has started under the supervision of M/s. Gherzi Eastern Ltd., the consultants for this project. The construction of Research Block is to be completed within 17 months.

8.9 राष्ट्रीय मलेरिया अनुसंधान संस्थान में राजभाषा विकास संबंधी गतिविधियां

संस्थान में राजभाषा हिन्दी के प्रयोग को बढ़ावा देने के उद्देश्य से अनेक गतिविधियों का आयोजन किया गया, जिसके अन्तर्गत हिन्दी कार्यशाला, विज्ञान दिवस पर वैज्ञानिक संगोष्ठी, हिन्दी सप्ताह के दौरान विभिन्न गतिविधियाँ आदि शामिल हैं। साथ ही राजभाषा अधिनियम के अनुपालन की दिशा में प्रशासन वर्ग के कर्मचारियों में राजभाषा के प्रयोग को बढ़ावा देने हेतु 'प्रोत्साहन योजना' लागू की गई एवं इसके पुरूस्कार हिन्दी सप्ताह के पुरूस्कार वितरण समारोह के अवसर पर प्रदान किए गए। यही नहीं राजभाषा विकास हेतु किए गए रचनात्मक प्रयास की ओर दृष्टिपात करें तो मलेरिया पत्रिका (तिमाही) के नियमित प्रकाशन के अलावा दो नए प्रकाशन—संदर्भ पुस्तिका एवं न्यूज लेटर (हिन्दी) भी प्रकाशित किए गए।

वर्ष के दौरान सर्वप्रथम दिनांक 30 मई 2005 को निदेशक महोदय की उपस्थिति में प्रशासन वर्ग के कर्मचारियों हेतु एक हिन्दी कार्यशाला का आयोजन किया गया जिसमें मुख्य अतिथि एवं वक्ता के रूप में स्वास्थ्य एवं परिवार कल्याण मंत्रालय के निदेशक श्री कमल सिंह को आमंत्रित किया गया था। श्री सिंह ने राजभाषा नीति, नियम, अधिनियम विषय पर जानकारी देते हुए कर्मचारियों से चर्चा की और बताया कि एक सरकारी कर्मचारी होने के नाते हमारा यह दायित्व है कि हम सरकार की राजभाषा नीति का पालन करते हुए अपना सरकारी कामकाज राजभाषा हिन्दी में करें।

यहां यह भी उल्लेखनीय है कि सितम्बर माह में हिन्दी दिवस 14 सितम्बर 2005 के उपलक्ष्य में हिन्दी पखवाड़ा मनाया गया जिसमें दो गतिविधियाँ एक प्रशासन वर्ग के कर्मचारियों के लिए हिन्दी कार्यशाला और दूसरी वैज्ञानिकों के लिए वैज्ञानिक संगोष्ठी का आयोजन किया गया। इन दो गतिविधियों के अलावा चार अन्य प्रतियोगिताएं—टिप्पण-प्रारूपण

प्रतियोगिता, निबंध प्रतियोगिता तथा कर्मचारियों और अधिकारियों के लिए पृथक-पृथक वाद-विवाद प्रतियोगिताओं का आयोजन किया गया। इन प्रतियोगिताओं में संस्थान के अधिकारियों एवं कर्मचारियों ने अत्यंत उत्साहपूर्वक भाग लिया क्योंकि यह एक ऐसा पखवाड़ा था जिसमें आयोजित की गई गतिविधियों में किसी न किसी रूप में संस्थान के छोटे से छोटे कर्मचारी एवं बड़े से बड़े अधिकारी को शामिल किया गया था। संबधित प्रतियोगिताओं का आयोजन संस्थान के निदेशक प्रो. ए.पी. दाश के निदेशन में राजभाषा कार्यान्वयन समिति के विभिन्न सदस्यों द्वारा किया गया।

दिनांक 23 सितम्बर 2005 को पखवाड़े की अंतिम गतिविधि वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) और पुरूस्कार वितरण समारोह का सफलतापूर्वक आयोजन किया गया। इस अवसर पर प्रतियोगिता के निर्णायक के रूप में जाने-माने साहित्यकार एवं केन्द्रीय हिन्दी निदेशालय में निदेशक रह चुके डॉ. गंगा प्रसाद विमल एवं डा. अनिता, वरिष्ठ प्राध्यापक, राजधानी कॉलेज को आमंत्रित किया गया था। संबंधित समारोह में सर्वप्रथम वाद-विवाद प्रतियोगिता (अधिकारी वर्ग) का आयोजन किया गया, जिसमें 12 अधिकारियों एवं वैज्ञानिकों ने भाग लिया, जिसका विषय था 'शाकाहार बनाम मांसाहार'। उल्लेखनीय है कि राजभाषा कार्यान्वयन को बढ़ावा देने की दिशा में किया गया एक रचनात्मक प्रयास 'राजभाषा कार्यान्वयन संबंधी संदर्भ पुस्तिका' का विमोचन एवं प्रर्दशन भी इसी दिन क्रमशः सुविख्यात वैज्ञानिक प्रो. आर.सी. महाजन एवं संस्थान के निदेशक, प्रो. ए.पी. दाश द्वारा किया गया। इस पुस्तिका के अंतर्गत राजभाषा के प्रयोग संबंधी नियमों को संक्षेप में देने के साथ ही कुछ चुनिंदा प्रशासनिक शब्दों एवं टिप्पणियों को समाविष्ट किया गया है।

संदर्भ पुस्तिका के विमोचन के पश्चात सप्ताह के दौरान आयोजित विभिन्न प्रतियोगिताओं के पुरूस्कार प्रो. गंगा प्रसाद विमल, निदेशक प्रो. ए.पी. दाश, प्रो. आर.सी. महाजन एवं संस्थान के वरिष्ठ अधिकारियों डा. एम.ए. अंसारी एवं डॉ. अरूणा श्रीवास्तव के कर-कमलों से प्रदान किए गए।

यहाँ यह बताना प्रासांगिक होगा कि संदर्भ पुस्तिका के अलावा राजभाषा विकास की दिशा में एक न्यूज़ लेटर (हिन्दी) का विमोचन संस्थान के भवन निर्माण के शिलान्यास के अवसर पर परिषद मुख्यालय के महानिदेशक प्रो. एन.के. गांगुली के कर-कमलों से किया गया। वस्तुतः यह न्यूज लेटर, द्विभाषी रूप (अंग्रेजी-हिन्दी) में आरंभ किया गया है और इसमें मलेरिया संबंधी समाचार एवं अनुसंधान के साथ-साथ संस्थान के वैज्ञानिकों द्वारा किए गए अनुसंधान कार्य, अध्ययन अवसर, उपलब्धियों आदि को समाहित करके गागर में सागर भरने का कार्य किया गया है।

इसके अलावा 28 फरवरी 2006 को विज्ञान दिवस के उपलक्ष्य में वैज्ञानिकों हेतु एक वैज्ञानिक संगोष्ठी का आयोजन किया गया जिसमें मुख्य अतिथि एवं वक्ता के रूप में एन.टी.पी.सी. के महाप्रबंधक श्री मथुरा प्रसाद को आमंत्रित किया गया। संस्थान के निदेशक एवं प्रो. आर.सी. महाजन की अध्यक्षता में श्री मथुरा प्रसाद ने 'पर्यावरण एवं उसका स्वास्थ्य पर प्रभाव' विषय पर स्लाइडों के माध्यम से अपने विचार प्रस्तुत किए एवं वैज्ञानिकों के साथ तर्क-वितर्क किया। विज्ञानीय-स्तर पर राजभाषा को प्रतिष्ठित करने के इस प्रयास का वैज्ञानिकों ने भरपूर लाभ उठाया क्योंकि श्री प्रसाद ने इसे सामान्य किन्तु गूढ विषय को समग्रता में लेते हुए पर्यावरण के एक-एक पहलू से अवगत कराया। श्री प्रसाद ने अत्यंत सहज एवं सरल हिन्दी में जीवन के प्रत्येक पहलू में पर्यावरण की महत्ता को समझाया। संस्थान में ही नहीं वरन् संस्थान की क्षेत्रीय इकाइयों में भी राजभाषा कार्यान्वयन के प्रति रूचि जागृत करने के उद्देश्य से हिन्दी

दिवस के उपलक्ष्य में विभिन्न प्रतियोगिताओं का आयोजन किया गया। जिनमें मुख्य हैं-

जबलपुर क्षेत्रीय इकाई जहां कि निबंध प्रतियोगिता तथा वाद-विवाद प्रतियोगिता का आयोजन हर्षोल्लास के साथ किया गया। इन प्रतियोगिताओं में कार्यालय के अनेक कर्मचारियों ने भाग लिया। वाद-विवाद प्रतियोगिता का विषय था 'देश में पाश्चात्य संस्कृति का विस्तार भारतीय समाज के लिए घातक है' या 'आधुनिक युग में व्यक्तित्व के समग्र विकास के लिए हिन्दी का ज्ञान आवश्यक है'। इस प्रतियोगिता में प्रथम, द्वितीय, एवं तृतीय विजेता कर्मचारी क्रमशः श्री प्रवीण कुमार कुंड, श्री सुधीर कुमार सेन, श्री पुष्पेन्द्र तिवारी थे। वाद-विवाद प्रतियोगिता के अलावा निबंध प्रतियोगिता का भी आयोजन किया गया था जिसका विषय था 'जन-संचार माध्यमों का जनसमस्याओं के निवारण में योगदान'। जिसमें प्रथम, द्वितीय, तृतीय क्रमशः श्री अजय सक्सेना, श्री इंतजार अहमद सिद्दीकी, श्री हीरालाल चौधरी रहे। इन दोनों प्रतियोगिताओं के पुरूस्कार डॉ. नीरू सिंह, प्रभारी अधिकारी, क्षेत्रीय इकाई के कर-कमलों से प्रदान किए गए। जबलपुर ही नहीं वरन् संस्थान की शाहजहांपुर क्षेत्रीय इकाई में भी राजभाषा के प्रयोग को बढावा देने के उदेश्य से निबंध प्रतियोगिता एवं भाषण प्रतियोगिताओं का आयोजन किया गया। इन प्रतियोगिताओं में भी कार्यालय के अनेक कर्मचारियों ने उत्साहपूर्वक भाग लिया। दोनों प्रतियोगिताओं का संचालन डॉ. हरदेव प्रसाद गुप्ता, सहायक अनुसंधान अधिकारी द्वारा किया गया। निबंध प्रतियोगिता के प्रथम, द्वितीय, तृतीय विजेता प्रतियोगी क्रमशः श्रीमती अर्चना गुप्ता, श्री उदयवीर सिंह, श्री विजय ओम गुप्ता थे। निबंध प्रतियोगिता के अलावा भाषण प्रतियोगिता का आयोजन भी इसी दिन किया गया। जिसमें विजेता प्रतियोगी क्रमशः श्री विजय ओम गुप्ता, श्री विनोद कुमार सिंह श्री नरेन्द्र शुक्ला रहे। अंततः दोनों प्रतियोगिताओं के पुरूस्कार वितरण हेतु समारोह का आयोजन किया गया और जिसमें अध्यक्षीय भाषण देते हुए प्रभारी अधिकारी डॉ. गुरूदेव प्रकाश दत्ता ने राष्ट्र भाषा की महत्ता पर प्रकाश डालते हुए विजेता प्रतियोगियों के पुरूस्कार की घोषणा की। अतः कहा जा सकता है कि संस्थान में राजभाषा के प्रयोग के प्रति क्षेत्रीय ईकाईयाँ भी अत्यंत सचेत एवं जागरूक रहकर राजभाषा नियम-अधिनियम के अनुपालन की दिशा में सक्रिय भूमिका निभा रही है।

इस प्रकार यह कहने में कोई अतिशयोक्ति नहीं होगी कि वर्ष 2005-06 के दौरान संस्थान एवं क्षेत्रीय इकाईयों में राजभाषा के प्रयोग को बढ़ावा देने हेतु सृजनात्मक, रचनात्मक एवं व्यावहारिक कार्य एवं कार्यक्रमों के माध्यम से हर संभव प्रयास किया गया। संस्थान एक विज्ञानीय अनुसंधान संस्थान होने के बावजूद राजभाषा नियम अधिनियमों का अनुपालन करते हुए राजभाषा के प्रयोग को बढावा देने में प्रत्येक कोण से प्रयासरत है और इसका साक्षात प्रमाण राजभाषा संबंधी गतिविधियों का उल्लेखित सारांश हैं जो कि इसके बहुमुखी विकास का प्रतिरूप है।

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Names are listed in alphabetical order by surname; Staff position as on 31 March 2006. (*Up to 22 February 2006; #Up to 1 December 2005; Up to 5 October 2005)

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