ENTOMOLOGY

VECTOR BIOLOGY

Anopheles culicifacies Complex

Bionomics and Distribution Pattern of Members

Anopheles culicifacies collected from highly malarious villages in the District Gadchiroli (Maharashtra) were cytologically examined for sibling species composition. Results revealed that species B and C were sympatric in these villages with predominance of species C (>80%), an established vector of malaria. Similarly, in study villages of Districts Kanker and Bastar (Chhattisgarh) species B and C were sympatric with predominance of latter. In District Hazaribagh (Jharkhand) species A, B and C were sympatric in study villages but species B was predominant comprising 69.4% of the total identified. In Karnataka, cytological examination of *An. culicifacies* samples from study villages of District Bijapur revealed prevalence of species A and B, the former being predominant comprising 88.9%, whereas in District Dharwad only species B was found prevalent in the study villages. In District Udaipur (Rajasthan) species A and B were found sympatric and species A was polymorphic for i^1 inversion. In all the above mentioned districts *An. culicifacies* sibling species were primarily zoophagic.

Molecular Diagnostic Assays for the Identification of Members

The two regions, intertranscribed sequence 2 (ITS2) of rDNA and cytochrome oxidase II (COII) of mitochondrial DNA were analyzed to find species-specific variations to differentiate the so far reported five sibling species of *An. culicifacies* complex. The ITS2 amplicon (~ 500 bp) digested with *Rsa* I could differentiate the five sibling species into two groups—A/D from B/C/E group (Fig. 1). DNA sequencing of these two amplicons (ITS2 and COII) and their se-

HIGHLIGHTS

- In *An. culicifacies* complex, sequence alignment of COII region was utilized to design primers that could differentiate all the five species in two step PCR assays. Microsatellite markers isolated from *An. culicifacies* species A were used for genotyping species A populations from different geographical areas.
- Allele-specific PCR assay developed to differentiate members of *An. fluviatilis* complex was validated using cytologically identified specimens. The results of PCR assay were found to be in agreement with those of cytotaxonomy.
- An album of GIS predicted distribution of anopheline species in India has been produced in form of a CD. It also contains blowup maps of GIS predicted district-wise favourable areas and the validation of GIS predicted distribution through reported surveys.
- Field trials revealed that indoor residual spraying of bendiocarb was highly effective in controlling the densities of DDT and HCH resistant *An. culicifacies*.
- Field evaluation of Agnique MMF (a monomolecular surface film) and Triflumuron (an insect growth regulator) revealed their effectiveness against immature stages of vector mosquitoes.

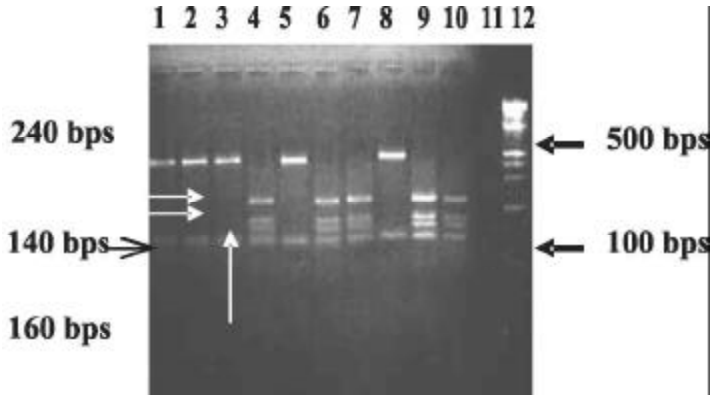

Fig. 1: Rsa I RFLP in ITS2 region of rDNA among the *An. culicifacies sensu lato* collected from Jabalpur. Lanes 1,2,3,5 & 8: Species C; Lanes 4,6,7,9 & 10: Species D; Lane 11: Negative control; and Lane 12: 100 bp ladder

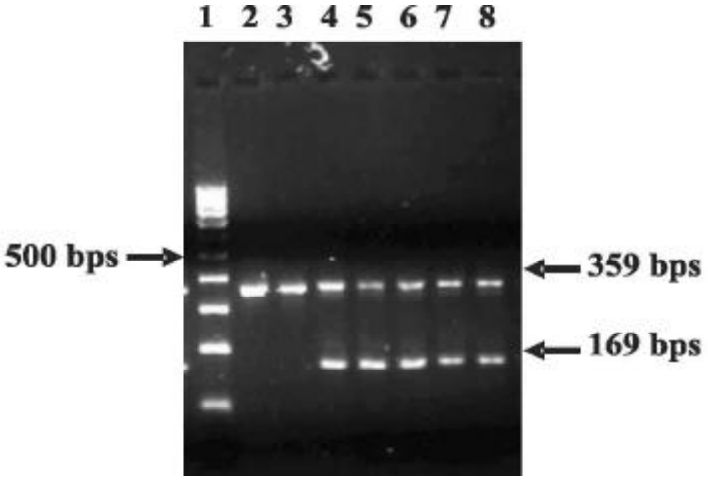

Fig. 2: Result of allele-specific PCR assay to differentiate species A and D among the *An. culicifacies* complex with the primers designed from COII region of mtDNA. Lane 1: 100bp ladder; Lanes 2&3: Species A; Lanes 4–8: Species D from Jabalpur

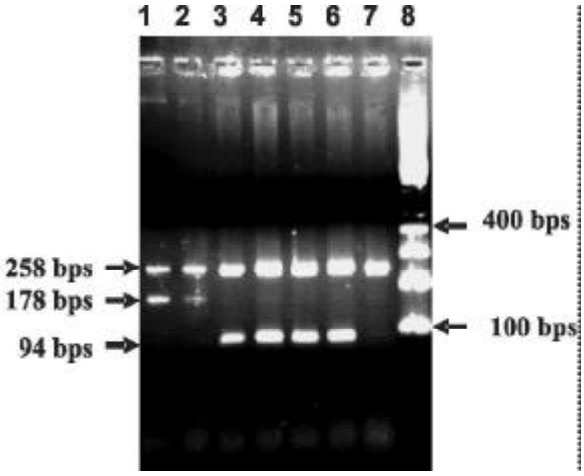

Fig. 3: Result of allele-specific multiplex PCR assay to differentiate species B and E among the *An. culicifacies* complex with primers designed from COII region of mtDNA. Lanes 1–2: Species E from Rameswaram; Lane 3–6: Species C from Bastar; Lane 7: Negative control; and Lane 8: 100 bp marker

quence alignment showed species-specific differences. The ITS2 was not used for primer designing to differentiate the species as the primers for this differentiation have already been designed and evaluated from D3 and D2 regions of 28S rDNA (Annual Report 2001). However, the sequence alignment of COII was utilized to design primers that could differentiate all the five species in two PCR assays on the pre-grouped A/D and B/C/E species by D3/D2-PCR assay. The approach followed for differentiating all the five members of *An. culicifacies* complex was: First—D3/D2 PCR assay to differentiate A/D from B/C/E; Second—A-D-PCR to differentiate species A from species D (Fig. 2); and Third, B-C-E-PCR to differentiate the three species, B from C from E (Fig. 3). These PCR assays are being validated for field use.

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

This year 14 new microsatellite markers were isolated from *An. culicifacies* species A and thus the total number of microsatellite markers isolated by Malaria Research Centre, for this species, has increased to 31. Three populations of *An. culicifacies* species A from Districts Kheda (Gujarat), Sonepat (Haryana) and Bijapur (Karnataka) were genotyped using 10 most promising microsatellite markers and two populations from Districts Allahabad (U.P.) and Udaipur (Rajasthan) were genotyped using five microsatellite markers.

Anopheles fluviatilis Complex

Distribution, Bionomics and Biology of Sibling Species

Mapping of the geographical distribution of *An. fluviatilis* sibling species continued. Samples examined from Districts Mandya and Gulbarga (Karnataka) revealed the prevalence of only species T in these districts which was found polymorphic for q^1 inversion. *An. fluviatilis*

collected from Iran Shahr, Baluchistan (Iran) were also examined and species T was found prevalent in this area.

Cytological examination of *An. fluviatilis* population from villages under Laksar PHC of District Hardwar (Uttaranchal) revealed the existence of a new inversion homozygote in this area. The break points for new inversion were identified and the inversion genotype on chromosome arm 2 was observed as $+q^1r^1s^1$. Collections made during summer (April–May) and post-monsoon season (September–December) from villages Dargahpur and Ismilepur showed prevalence of this new cytological variant in sympatric association with species T and U. A total absence of inversion heterozygotes between them suggests the possible existence of a new species in *An. fluviatilis* complex. It is noteworthy to mention that PHC Laksar contributes bulk of malaria cases in the district with *An. fluviatilis* in highest proportion (36%) among 13 anopheline species recorded from this area. Therefore, studies have been initiated to resolve the taxonomic status of the new cytological variant and explore its role in malaria transmission.

Efforts were made to establish cyclic colonies of species S and U. Short-term cultures of species U could be established and bidirectional reciprocal crosses were made between species T and U. The hatchability ranged from 70–83%. In both the crosses the F_1 hybrid females were found with normal reproductive organs but the hybrid males were sterile with testis devoid of sperms. These observations showed that apart from pre-mating barriers there exist post-zygotic barriers between species T and U.

Molecular Characterization of Members of *An. fluviatilis* Complex—Development of Species-specific Markers and Microsatellite Markers (ICMR-Genomics Project)

Validation of diagnostic PCR assay for the identification of members: In the year 2001, a PCR assay was developed for the differentiation of members of *An. fluviatilis* complex, which is based on the sequences of D3 domain of 28S rDNA. The PCR assay was tested against over 700 mosquitoes collected from different parts of India (Districts–Hardwar, Udham Singh Nagar, Sundargarh, Malkangiri, Koraput, Gulbarga and Mandya) having different sympatric associations. Among these 147 specimens were also examined chromosomally for the validation of PCR assay. The PCR assay was found to differentiate unambiguously all the members of the complex and the results of PCR assay were in agreement with that of cytotaxonomy in the areas where fixed diagnostic inversions are present. In District Mandya of Karnataka, where q^1 inversion polymorphism exists—heterozygotes ($q^1/+^{q1}$) were found in Hardy-Weinberg equilibrium, all specimens were identified as species T. In the absence of chromosomal marker, this population was considered as species T due to resemblance with species T in biological characteristics—zoophagy and resting in cattlesheds.

Anopheles minimus Complex

Molecular Characterization of *An. minimus* from Assam

Based on 28S-D3 rDNA sequences of *An. minimus* species A and C, primers were designed for the differentiation of species A and C. The primers tested against *An. minimus* were collected from Districts Dibrugarh and Sonapur of Assam. Out of 20 samples tested, all were

identified as species A. To confirm the result, two samples of each population mentioned above were sequenced. The sequenced data confirmed that these mosquitoes were indeed species A.

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

GIS Based Distribution of *An. culicifacies* in India

An. culicifacies is widely distributed throughout the country except in Andaman and Nicobar Islands. It is abundant in the plains but less prevalent in eastern part of India. Though the species is of the plains but also reported from higher altitudes—Nainital (1600 m), Kashmir (3000 m) etc. Therefore, from the altitude layer 0–3000 m was selected as the base layer and the most favourable range was taken as 0–1350 m with temperature > 20°C. Rainfall is an important factor in regard to *An. culicifacies*, the species being monsoon-associated and the maximum breeding occurs after the rains. High rainfall areas ≥ 2400 mm and very low rainfall areas ≤ 200 mm were deleted. Maps were integrated and the resulting map is shown in Fig. 4.

Blowup of distribution of *An. culicifacies* in northeastern India is shown in Fig. 5. The dots in the districts indicate the reported distribution of the species. In all districts where the species has been reported, GIS also predicts favourable areas for its distribution.

GIS Mapping of *An. minimus*

The resultant map after integration of thematic maps—soil, forest cover, rainfall, temperature and altitude using GIS mapped the areas favourable for *An. minimus.* Surveys were conducted to validate the results in favourable and unfavourable areas by our team as well as by an independent team and sites were selected both from reported and nonreported areas. Surveys were conducted in four states—Uttaranchal in north, West Bengal, Assam and Meghalaya in northeast. In the northeast a stretch of 900 kms was covered. Amazingly, *An. minimus* were collected from all locations in GIS predicted favourable zone (Table 1). In two districts—Champawat, Banbasa areas of Uttaranchal and Dhubri of Assam, in the former, the species was reported to have disappeared after 1950s, and latter, it was not reported in earlier entomological surveys, during present surveys in both the places *An. minimus* was encountred (Malaria Research Centre—Annual Reports). Thus besides validation of GIS prediction, reappearance of *An. minimus* at Banbasa and first report from Dhubri was established.

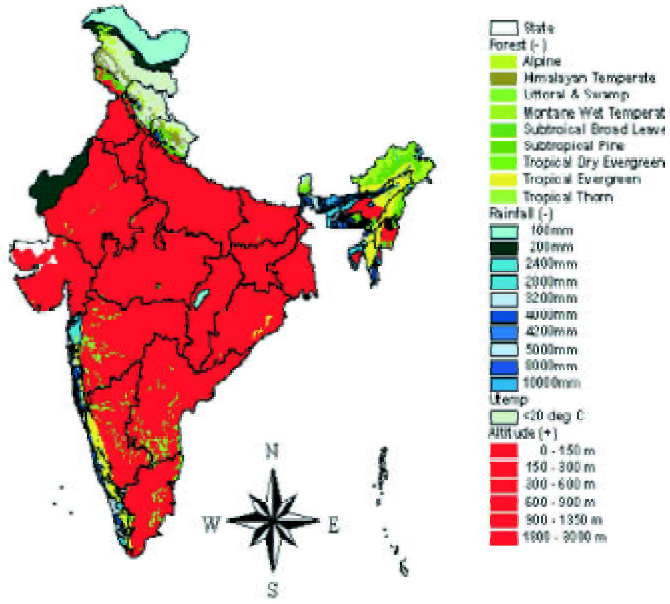

Fig.4: GIS predicted distribution of *An. culicifacies* subject to condition, the area being an rural/urban area

GIS predicted precisely the locations in these districts to conduct entomological surveys and the species could be found there. Blind survey was conducted by independent team in both favourable and unfavourable areas. In favourable areas the species was found and in unfavourable areas on the border of Karbi-Anglong, it was found to be absent.

Using GIS, percentage of favourable area for distribution of *An. minimus* in different states was estimated. It showed that most of the area in northeastern states was favourable for *An. minimus*. In Mizoram favourable area is about 90.61%, and in Manipur, Nagaland, Tripura and Assam 70, 35, 33 and 25% respectively. In other states it was less than 10% except Kerala. There are some favourable areas in Kerala and Maharashtra, till date there is no report of *An. minimus* from these areas. Most favourable corridors for distribution of *An. minimus* were also mapped by stratifying favourable

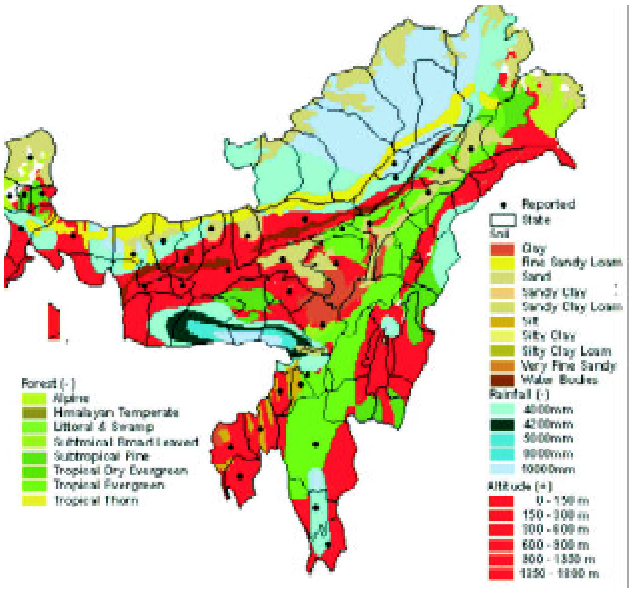

Fig.5: Blowup of distribution of *An. culicifacies* in northeastern India, the dots represent the districts, where *An. culicifacies* was reported. Red colour shows areas favourable for distribution in that particular district

Table 1. Validation of *An. minimus* in GIS predicted areas by precision surveys

Collection site/ District/State	Period of survey	MHD* of *An. minimus*	Larval density**	Remarks
Banbasa, Uttaranchal	May 2001	0.25	0.00	Reappearance of species
	Jul 2001	0.53	0.02	
	Aug 2001	0.73	0.03	
Jalpaiguri, West Bengal	Oct 2001	1.70	0.08	
Dhubri, Assam	Oct 2001	0.91	0.06	Reported first time
Kamrup, Assam	Oct 2001	21.80	1.40	
Barpeta, Assam	Oct 2001	Not done	0.18	
Burnihat, Meghalaya	Oct 2001	1.16	Not done	
Shillong, Meghalaya	Oct 2001	0.33	Not done	
Darrang, Assam	Jun/Jul 2001	4.00		
Goalpara, Assam	Sep 2001	21.00		
Karbi-Anglong, Assam	Oct 2002	0.00		*An. minimus* was not found in GIS predicted unfavourable area

*MHD: No. of mosquitoes collected per man per hour; **Larval density—No. of larvae per dip.

Table 2. GIS predicted most favourable corridors for *An. minimus*

Category	Altitude (m)	Rainfall (mm)	Temp. (°C)	Forest cover
Category 1 (Most favourable)	0–600	2000–2800	22.5–25	Evergreen
Category 2 (Medium favourable)	600–900	2800–3200	20–22.5	Moist deciduous
Category 3 (Less favourable)	900–1800	3200–4000	< 20	Moist deciduous

The species population is likely to be most stable in category 1 and least in category 3.

zones in high, medium and low categories. The stratification was based on forest cover, temperature, rainfall and altitude, range identified for three zones is given in Table 2.

An. minimus in Banbasa (Uttaranchal)

To validate the GIS prediction, *An. minimus* were collected from Banbasa area of Uttaranchal state though it has been reported to have disappeared from this area in early 50's. The surveys were conducted in post-monsoon (October and November 2001), pre-monsoon (March, April and May 2002) and monsoon (June-July 2002) seasons, per man hour density of the species was recorded as 0.9, 0.26 and 0.67 and the larval density per dip as 0.5, 0.2 and 0.4 respectively. The larvae collected from the streams were reared individually in the laboratory till adult emergence. Individual specimens of larval and pupal skins were mounted and examined for the identification characters. The most important character of *An. minimus* larvae is the presence of branched seta-0 on abdominal segments III–VI, and this character was recorded in 22 mounted slides prepared in different seasons, which confirms the presence of *An. minimus* in Banbasa, a GIS predicted area.

GIS Based Distribution of *An. stephensi* —An Urban Vector

An. stephensi is found throughout the country except in Andaman and Nicobar Islands and higher altitudes. It is responsible for transmission of malaria in urban/peri urban areas of the country but the recent epidemics of malaria in Jodhpur and Bikaner districts of Rajasthan were attributed to this species. *An. stephensi* breeds in domestic containers such as tanks, cisterns, coolers, wells, etc. It has also been found breeding in irriga-tion channels and in rice fields. Distribution of *An. stephensi* is shown in Fig. 6, subject to the condition, the area is urban/peri urban. Altitude range from 0–900 m, temparature >20°C and rainfall < 2400 mm have been taken as favourable.

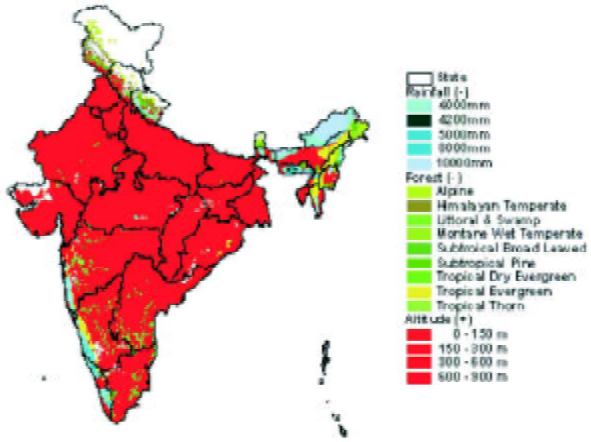

Fig. 6: GIS predicted distribution of *An. stephensi*, subject to the condition, the area being an urban/peri urban area

Besides mapping distribution of major malaria vectors, all 58 anophelines have been mapped using GIS and a CD has been produced consisting of an album with the objective to make ready to use product. This album consists of 58 maps each

showing the GIS predicted distribution of anopheline species in India, along with the blowup maps of GIS predicted district-wise favourable areas and the validation of GIS predicted distribution through reported surveys (Fig. 7).

The technique can delineate the areas favourable for any species of flora and fauna, which is very useful for precision surveys. The technique is fast and can be easily duplicated at desired scale in other parts of the country/world. Since this unique technique identifies the location for precision surveys in an area where the species is likely to be found, thus location wise control activity may be implemented which may be effective in terms of results and cost.

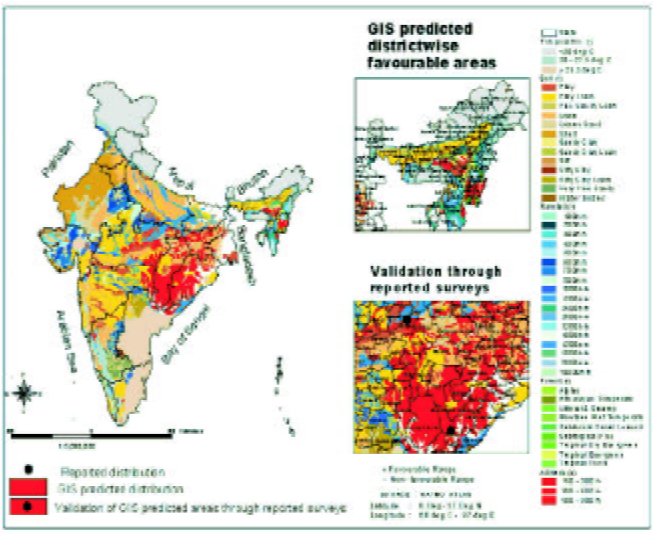

Fig. 7: GIS predicted distribution of *An. sergentii* in India, a blowup showing northeastern states and validation through reported distribution is also depicted in insets

Spiracular Index and Bioecology of *An. minimus* in Kamrup District, Assam

A total of 1811 *An. minimus* were collected from 12 villages of Kamrup district, Assam, located in evergreen-forested zone in pre-monsoon (April–May 2002) and post-monsoon (September–October 2001) season. Out of 1811 *An. minimus*, 665 (MHD 15.7) were collected in pre-monsoon and 1146 (21.8 MHD) in post-monsoon season. During pre-monsoon and post-monsoon 410 and 310 specimens were dissected respectively for spiracular index. In pre-monsoon, the average spiracular length of *An. minimus* was 0.085 mm (± 0.01), average thorax length was 0.94 mm (± 0.05) and spiracular index was 9.04 (± 0.74) whereas in post-monsoon average spiracular length was 0.08 mm (± 0.01), average thorax length was 0.94 mm (± 0.07) and spiracular index was 9.41 (± 0.70) as calculated by using the technique and formula of Vinogradaskaya 1969. *An. minimus* population does not show any seasonal fluctuation in their spiracular index ($p < 0.05$). In pre-monsoon the humidity recorded was 75–81% whereas in post-monsoon the humidity was 72–95%.

It may be noted that *An. minimus* is a small sized mosquito of wet zone as it measures 2–3 mm as compared to *An. stephensi* type form 5–6 mm and *mysorensis* 4–5 mm of arid zone—the xerophilic species. The spiracular index of *An. minimus* was higher 9.4 as compared to that of *An. stephensi* type form 9.09 and *mysorensis* 7.69. This establishes that *An. minimus* is a hygrophilic species.

Bioecology

Breeding habitat: *An. minimus* was found breeding in slow moving streams with grassy margins. A total of 575 larvae were collected and identified. Out of these 235 were identified

as *An. minimus* and others were associated species—*An. splendidus, An. jeyporiensis* and *An. maculatus*. It is noteworthy to mention that the breeding was observed in those streams which were near to the houses (< 1000 m). Fifteen *An. minimus* emerged from the larvae collected from rice fields and small irrigation channels adjacent to the houses.

Resting collection: Searches made inside the houses and outdoor shelters yielded 665 specimens of the species. Out of these 640 (96.24%) accounted for indoor collection during pre-monsoon season (April–May). Similarly, during post-monsoon collection (September–October) out of 580 total collected 565 (97.41%) were from indoors. The preferred indoor resting sites included under surface of the furniture, mosquito net on the bed, hanging clothes, cobwebs and walls. The most preferable resting site was under surface of the furniture in both the seasons—pre-monsoon and post-monsoon as 290 (60%) and 245 (55%) specimens were collected respectively.

Indoor Resting Behaviour of *An. stephensi* in an Arid Zone (District Jodhpur, Rajasthan)

Success of malaria control by IRS largely depends on resting behaviour of the vector mosquitoes on sprayable surfaces. Endophilic (indoor resting) and endophagic (indoor feeding) species are known to be highly vulnerable to this strategy. The success rate with exophilic (outdoor resting) and endophagic species depends on the time spent by the species on sprayable surfaces during pre- and post-biting rest. Therefore, studies on resting behaviour of *An. stephensi* were carried out to assess its amenability to control through indoor residual spray in an Arid zone of District Jodhpur, Rajasthan during March–April 2002. In the present study, resting behaviour of the species during all its movement rhythms covering 24 hours period related to: (i) swarming/mating; (ii) pre- and post-biting rest; (iii) after feed resting between hopping movements; (iv) night and daytime resting; and finally (v) diel activity movements in response to temperature changes were carried out in both unsprayed and spayed villages. Analysis revealed that about 95 and 97% of *An. stephensi* preferred to rest on unsprayable surfaces—household objects in unspyayed and spayed villages. The most preferred resting sites in both groups of villages under household objects were hanging clothes, utensils and cupboards. There was no significant difference in resting behaviour of the species in both groups of villages ($p>0.05$). Pre- and post-biting rest period ranged from 5–15 and 15–25 min respectively. After biting out door, species starts entering the rooms at around 2330 hrs. Maximum entries—56 and 62% of the species into the rooms were observed during third quarter (0100–0400 hrs) in unsparayed and sprayed villages respectively, and coincided with fall in ambient temperature indoor below 30^{O}C. Statistically there was no significant difference in the entry of mosquitoes ($p > 0.05$) in both the groups of villages. Therefore, control of *An. stephensi* in study area requires an integrated control strategy based upon intersectoral, environmental, larviciding with chemical/biolarvicide and use of larvivorous fish wherever feasible. Such a control strategy offers cost-effective and sustainable option than indoor residual spray.

VECTOR–PARASITE INTERACTION

Cyclical Transmission of Rodent Malaria

Laboratory cyclical transmission of rodent malaria parasites—*P. yoelii yoelii, P. chabaudi, P. berghei* and *P. vinckei* was revived in BALB/c mice using *An. stephensi* as vector. Cyclical transmission of *P. vinckei* was also maintained through other vectors—*An. culicifacies* species A and *An. fluviatilis* species T.

Immune Response of *An. stephensi* against *Micrococcus leutus*

Insects are known to mount a strong immune response against any invading parasite. Success of sporogony largely depends upon the ability of insect to combat the parasite and/or the ability of parasite to evade the immune response. Response of *Anopheles stephensi*, an urban malaria vector to bacterial infection and to sterile injury is studied in immature stages. Proteins being the first conceivable product of gene action are studied using SDS-PAGE as tool. Denaturating SDS-PAGE analysis revealed induction of a 42 kDa polypeptide in female pupae of urban malaria vector— *An. stephensi* upon infection with gram-positive bacteria *Micrococcus leutus*, that was absent upon septic injury. Septic injury seems to activate phenoloxidase cascade, as melanin formation is responsible for wound healing in insects. Our study identifies up-regulation of 35 and 65 kDa proteins in larvae and pupae both in whole body and tissue specific expression. It is worth mentioning here that serine proteases have molecular weight of 35 kDa whereas, phenoloxidases have molecular weight in the range of 60–70 kDa. Besides these five, polypeptides (20, 45, 52, 70 and 150 kDa) are also enhanced in their expression upon injury. Similary, studies on the immunity of refractory and susceptible strains of *An. culicifacies* in response to rodent malaria parasite and bacteria are in progress.

INSECTICIDE RESISTANCE

Present Status of Insecticide Resistance in *An. stephensi* from Delhi

Susceptibility tests against larvae and adult mosquitoes of *An. stephensi* strain collected from southDelhi revealed partial resistance in adult mos-quitoes to DDT and a very high degree of resistance to malathion and dieldrin, however, the mosquitoes were fully susceptible to other insecticides (Table 3). Among the larvicides tested, this strain was found to be highly resistant to fenthion but it was fully susceptible to teme-

Table 3. Susceptibility of *An. stephensi* strain from south Delhi to insecticides

Insecticide	% mortality after 24 h	Time in min	
		LT_{50}	LT_{90}
DDT (4%)	62.5	50.6	114.2
Malathion (5%)	5	6.54**	10.63**
Fenitrothion (1%)	79 (100)*	23.93	63.32
Propoxur (0.1%)	100	20.8	59.5
Deltamethrin (0.05%)	100	2.36	9.76
Lambdacyhalothrin (0.05%)	100	3.33	16.22
Cyfluthrin (0.15%)	100	4.11	14.17
Etofenprox (0.1%)	100	10.73	24.4

*Exposure time 2 h; ** Time in hours.

Table 4. Larval susceptibility of *An. stephensi* from south Delhi to some larvicides

Larvicides	LC_{50} (ppm)	LC_{90}
Temephos	0.0098	0.0199
Fenthion	>0.125	>0.125

phos (Table 4). Further, studies will be carried to monitor present status of susceptibility to various insecticides in the field populations of *An. stephensi* from Rajasthan; and resistance mechanism and inheritance pattern of resistant gene in this strain.

A New Focus of Malathion-resistant *An. culicifacies in* District Chhindwara, M.P.

Surveys were carried out in the villages of Mohkher block in District Chhindwara (Madhya Pradesh) in November and December 2001; and March 2002 and in the villages in Pandhurna block on the Madhya Pradesh-Maharashtra border in December 2001 and March 2002. In insecticide susceptibility tests the population was resistant to DDT (36–84%). To malathion Mohkher population was 14–40% resistant while in Pandhurna the resistance was in the range of 36–63%. Mohkher block was under regular indoor sprays of DDT while in Pandhurna block indoor spray in public health programme was discontinued about a decade ago. In both the areas malathion was never sprayed in public health programme. The observed resistance to malathion in *An. culicifacies* could be attributed to selection by the pesticides being used in agriculture on cash crops—cotton, vegetables, oil seeds, etc. Serial synergist-insecticide bioassays on the field populations indicated the involvement of malathion carboxylesterase as the major mechanism of malathion resistance though the involvement of mixed function oxidases as a secondary mechanism could not be ruled out.

VECTOR CONTROL

Follow-up Studies on the Impact of Indoor Residual Spraying of Bendiocarb 80% W.P. (Carbamate) against DDT and HCH Resistant Malaria Vector—*An. culicifacies* in Malaria Endemic Villages of Distt. Ghaziabad (U.P.) [Contract Research Project with M/s. Hoechst]

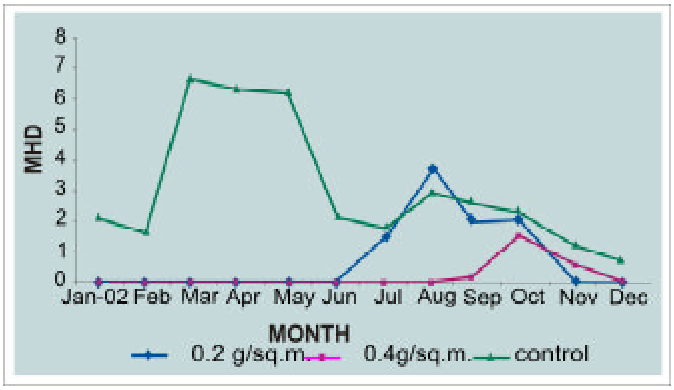

Fig. 8: Man hour density of *An. culicifacies* in selected villages of Distt. Ghaziabad, U.P.

Follow-up studies were carried out during the year to study the efficacy of bendiocarb in interrupting the malaria transmission. The SPR in village sprayed with bendiocarb @ 0.2 g/m^2 ranged from 0 to 6.4, in 0.4 g/m^2 bendiocarb sprayed village the SPR ranged from 0 to 4.5 where as in the control village the SPR ranged from 0 to 44.4. The SfR was nil in both the sprayed villages whereas in the control village few cases were reported. The study indicates that bendiocarb spraying has resulted in interruption of malaria transmission in the experimental villages when compared to the control village. Ento-

mological studies revealed that MHD of *An. culicifacies* was found to be in the range of 1.5–3.7 during the transmission season (July to October) in 0.2 g/m^2 bendiocarb sprayed village and in 0.4 g/m^2 bendiocarb sprayed village the MHD of *An. culicifacies* was in the range of 0–1.5 whereas in the control village it was in the range of 1.7–3.0 in the same period (Fig. 8). This clearly sug-gests that bendiocarb residual spraying is highly effective in controlling the mosquito densities.

Laboratory and Field Evaluation of Teknar HP-D (*B. thuringiensis* var *israelensis*) [Contract Research Project with M/s. Margo Bio-controls Pvt. Ltd.]

Efforts have been initiated to evaluate the bioefficacy of *Bacillus thuringiensis* against target species of mosquitoes. Preliminary laboratory results revealed that *Bti* formulation has broad-spectrum larvicidal activity against mosquitoes. Cent per cent mortality in *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* was observed in laboratory trials with Teknar @ 0.1 ppm. Field trials are in progress.

Field Evaluation of Mosquito Larvicide and Pupicide Agnique MMF in different Urban Habitats against Malaria Vector, *An. stephensi* [Contract Research Project under WHOPES]

Evaluation of Agnique MMF—Poly (Oxy-1, 2-ethanediyl), a-isoctane cyl-w-hydroxy1, a mono-molecular surface film, developed by M/s. Cognis Corporation was carried out in the representative breeding habitats of *An. stephensi* in urban areas of National Capital Region, Delhi. Study was carried out in two parts—simulated field conditions and natural field conditions.

In simulated field testing cement tanks of 1 x 1 x 1 m size were used and the efficacy of larvicide, effective doses and duration of effect against *An. stephensi* were determined. The impact of larvicide applied in different doses was studied by monitoring number of adults emerged in specially designed mosquito traps. The efficacy of the test larvicide was assessed by measuring the larval density in water storage cemented tanks and wells in natural field conditions. Results showed that in simulated field testing the Agnique MMF caused 100% inhibition of emergence of *An. stephensi* adults from water storage tanks upto one week at the application rate of 0.4, 0.6 and 1 ml/m^2 and > 95% up to second week at all the three doses. Dose of 1 ml/m^2 was effective up to third week when 99.5% inhibition of adult emergence was achieved.

In natural field trials the results of Agnique MMF in water storage tanks with breeding of *An. stephensi* and *An. subpictus*, the dose of 2 ml/m^2 was effective. The per cent reduction in late instars was more than 75 on Day 4 and 100% control was achieved in one week and remained up to second week. Results with MLO when treated @ 20 ml/m^2 also gave the same results. Hence, the results clearly indicated that dose of 2 ml/m^2 of Agnique MMF can be selected for the effective control up to two weeks period.

In wells the dose of 1 ml/m^2 gave about 75% control of late instars of *An. stephensi* on Day 2 and remained 100% up to two weeks. Agnique MMF was safe against larvivorous fish (*Gambusia affinis*) and notonectid bug (*Anisops sardae*). Since the Agnique MMF is odor-

less, invisible, monomolecular film and spreads rapidly across standing water, it could be one of the choices in the larval control programmes in urban areas.

Field Evaluation of Insect Growth Regulator—Triflumuron against Larvae of Mosquito Vectors [Contract Research Project with M/s. BAYER (India) Ltd.]

Efficacy of triflumuron, an IGR compound was carried out in the representative breeding habitats of *An. culicifacies* and *Cx. quinquefasciatus* in rural areas of north Delhi and in the district Sonepat of Haryana state. In small-scale fields trials triflumuron was sprayed at doses of 0.25, 0.5 and 1 ppm. Habitats selected for *An. culicifacies* were pools, ditches and paddy fields. For *Cx. quinquefasciatus* habitats selected were polluted drains, pools and cement tanks. Results showed the reduction in immatures in all the doses tested. At the dose of 1 ppm the pupal production was nil after one week which remained so even up to four weeks. The occurrence of delayed mortality in larvae indicated the effective developmental inhibition potential of this compound.

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

A nine-month multicentric study was conducted (July 2001 to March 2002) involving two other ICMR institutes—VCRC, Pondicherry and RMRIMS, Patna, Bihar. A common protocol was prepared for both entomological and parasitological evaluations. MRC evaluated the efficacy of indoor residual spray of DDT in six districts —Bareilly (Uttar Pradesh), Mandya (Karnataka), Chhindwara (Madhya Pradesh) and Kamrup (Assam) and of malathion in two districts—Kheda (Gujarat) and Hardwar (Uttaranchal). Indoor residual spray was conducted under the supervision of MRC staff to achieve the desired coverage. Evaluation was carried out during pre- and post-spray periods on different aspects—vector abundance, species composition, susceptibility status of mosquitoes to insecticides, disease prevalence, etc.

Results of the evaluation indicated effectiveness of DDT sprays against *An. minimus*, a principle vector in northeast states. *An. culicifacies* was found resistant to DDT in susceptibility tests in all the areas of study and DDT spraying was not found very effective in controlling this species. However, in some study areas this species exhibited excito-repellency against DDT for few weeks after spray registering a decrease in vector density while the results of evaluation against malathion indicated effectiveness of malathion sprays in controlling the vector—*An. culicifacies* in District Hardwar and in District Kheda the species exhibited differential susceptibility in different areas.

Laboratory (Phase-I) Evaluation of Phenthoate against Urban Mosquito Vectors—*Anopheles stephensi* and *Culex quinquefasciatus* [Contract Research Project with M/s. EID Parry India Ltd., Chennai]

A new organophosphorus insecticide phenthoate was tested against urban malaria vector *An. stephensi* and pest mosquito *Culex quinquefasciatus* in the laboratory to determine its efficacy against larvae and adult mosquitoes and to compare the results of its bioefficacy with that of

malathion, another organophosphorus insecticide which is being used at present as an adulticide. Mosquito colonies—*An. stephensi* and *Cx. quinquefasciatus* maintained in the insectary of MRC were used. The efficacy of phenthoate was determined against larvae and adult mosquitoes as per WHO recommended procedure. The results of bioefficacy against phenthoate are given in Tables 5–8. Phenthoate was more effective against *Cx. quinquefasciatus* larvae (LC_{50} = 0.0217 ppm) than *An. stephensi* (LC_{50}= 0.368 ppm). However, against adult mosquitoes of *An. stephensi*, phenthoate did not produce any mortality, when exposed to insecticide impregnated papers at a concentration of 4%, whereas against *Cx. quinquefasciatus*, complete mortality was observed even at 2% concentration (LT_{50}= 11.9 min).When the sus-

Table 5. Efficacy of phenthoate against III instar larvae of *An. stephensi* and *Cx. quinquefasciatus*

Mosquito species	Concentration in ppm		?²(df)
	LC_{50} (95% confidence limit)	Regression equation	
An. stephensi	0.368 (0.248–0.453)	Y = 3.129X ± 6.3579	0.755 (2)
Cx. quinquefasciatus	0.0217 (0.0132–0.0302)	Y = 2.0671X ± 5.7486	4.337 (3)

Table 6. Efficacy of phenthoate against adult mosquitoes of *An. stephensi* and *Cx. quinquefasciatus*

Mosquito species	% concentration		?² (df)
	LC_{50} (95% confidence limit)	Regression equation	
An. stephensi	> 4%	—	—
Cx. quinquefasciatus	0.33% (0.108–0.508)	Y = 2.2495X ± 6.08	1.376 (2)

Table 7. Efficacy of phenthoate 2% (lethal time) against *Cx. quinquefasciatus*

Mosquito species	LT_{50} (Time in min)	Regression equation	?² (df)
Cx. quinquefasciatus	11.98 (7.045–16.78)	Y = 2.1686X ± 2.66	2.239 (2)

Table 8. Comparative susceptibility of adult mosquitoes of four strains of *An. stephensi* against malathion and phenthoate

An. stephensi strain	Per cent mortality	
	Malathion 5%	Phenthoate 4%
Wild strain, Delhi	18	6.6
Mutant strain (Black larvae)	50	66.6
Mutant strain (Golden yellow larvae)	70	80
Mutant strain (White eye larvae)	86.6	100

ceptibility of *An. stephensi* to phenthoate was compared with that of to malathion, it showed cross-resistance to both the insecticides.

Prospecting of Botanical Pesticides (Screening of Bioactivity of Plant Extracts against Mosquitoes Particularly *Anopheles* spp) [DBT Funded Collaborative Project]

This study was undertaken as a part of a collaborative project funded by DBT included various laboratories as shown in Table 9. Bioactivity of various plant extracts/fractions/formulations received from five extracting laboratories was determined against mosquitoes particularly the malaria vector *An. stephensi* using standard protocol which included larvicidal, adulticidal and IGR activities, oviposition deterrency and mosquito repellency. During the reporting period 107 samples were received of which 50 samples have been screened for different activities as mentioned above. Of these 16 samples showed positive results for larvicidal activity and 8 samples showed adultcidal activity (Table 10).

Larvicidal Properties of Crude Aqueous Extract of Leaf of a Coded Plant – SP2

Larvicidal efficacy of the crude aqueous extract of the leaf of a coded plant – SP2 was tested against five species of mosquitoes of three genera—*An. culicifacies* species A and C, *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypt*i. The calculated lethal concentration for 50% mortality (LC_{50}) of the exposed larvae was in the range of 0.25 to 0.59 ml/L. The efficacy was in the order *Cx. quinquefasciatus* (0.25ml/L) > *An. culicifacies* species A (0.26) >*An. stephensi* (0.33) > *Ae. aegypti* (0.36) > *An. culicifacies* species C (0.59).

Table 9. Activities in various laboratories

Activity	Responsible laboratories
Collection, preservation and taxonomic identification of plants	RRL, Jammu RRL, Trivandrum NBRI, Bangalore
Extraction and fractionation of herbal products	IIT, Delhi FRI, Dehradun EID, Parry (India) Ltd., Bangalore RRL, Trivandrum RRL, Jammu
Bioassay testing of the efficacy against agricultural pests	IHBT, Palampur EID, Parry (India) Ltd., Bangalore
Bioassay testing of the efficacy against mosquitoes *(An. stephensi)*	MRC, Delhi and Hardwar
Formulation of the selected samples	IIT, Delhi IPFT, Gurgaon

Table 10. Bioactivity of plant extracts against *An. stephensi*

S. No.	Sample code number	Bioactivity of plant extract		
		Larvicidal	Adulticidal	Repellency
1.	NBDB(4)-002B-07-P-13a	(+)	(–)	(–)
2.	NBDB(4)022B-08-P-10a	(+)	(–)	(–)
3.	NBDB(4)023B-07–P-10a	(+)	(–)	(–)
4.	NBDB(4)042B-07-P-10a	(+)	(–)	(–)
5.	NBDB(2)008A-06-P-01a	(+)	(+)	(+)
6.	NBDB(2)010A-06-P-10a	(+)	(–)	(–)
7.	NBDB(2)010A-06-P-10b	(+)	(–)	(–)
8.	NBDB(4)-001B-08-P-13a	(+)	(–)	(–)
9.	NBDB(3)-022i-08-P-11e1	(+)	(–)	(–)
10.	NBDB(2)-005A-07-P-10a	(+)	(+)	(–)
11.	NBDB(2)-005A-07-P-10b	(+)	(+)	(–)
12.	NBDB(2)-005A-07-P-10c	(+)	(+)	(–)
13.	NBDB(2)-005A-07-P-04a	(+)	(+)	(–)
14.	NBDB(2)-005A-07-P-04b	(+)	(+)	(–)
15.	NBDB(2)-005A-07-P-04c	(+)	(+)	(–)
16.	NBDB(2)-055D-11-P02oil	(+)	(+)	(–)

OTHER STUDIES

Operational Evaluation of the Stability of Iodine in Double Fortified Salt – A Multicentric Study (ICMR – NIN – MRC – RMRC, Dibrugarh – RMRC, Bhubaneswar–TRC – IRR)

The stability of iodine in double fortified and iodised salt during storage for a period of one year under programmatic condition was determined as a part of a multicentric study under ICMR. Four types of coded salt samples (one thousand kilogram each, 25 kg x 40 bags) double fortified with iron and iodine (DFS) and fortified with iodine alone (IS) in the two forms—refined common salt (RCS) and common salt (CS), produced from the factory were received at MRC in October 2001 and stored at three places— inside room, in verandah and outside conditions as per protocol. Within a month of the date of arrival of the salt samples at MRC, one packet from each bag was taken out randomly and repacked in three packets of 100 g each after remixing. One of these samples was sent to NIN and second sample was stored at MRC. The third sample was analyzed for iodine content at MRC laboratory. The same process was repeated after 3 months, 6 months, 9 months and 12 months of the date of production of salt samples. Results of iodine content in the coded salt samples are given in Table 11. It was observed that the iodine content in the salt samples with yellow and green colour packing remained stable over a period of one year even

Table 11. Summarized results of iodine estimation in different types of salt samples RCS/ CS/ DFS/ IS* stored under different conditions over a period of one year at Malaria Research Centre

S. No.	Mean ± S.D.				
	Initial stage (0)	3 Months	6 Months	9 Months	12 Months
Y-1 to 15	44.01 ± 11.83	46.7 ± 9.48	37.21 ± 15.73	41.53 ± 11.87	41.5 ± 11.4
Y-16 to 20	43.92 ± 12.08	41.05 ± 10.11	35.01 ± 11.87	35.56 ± 11.28	39.48 ± 12.8
Y-21 to 40	51.69 ± 9.68	47.95 ± 8.36	45.10 ± 10.24	40.07 ± 10.16	40.6 ± 7.1
B-1 to 15	23.61 ± 10.54	26.06 ± 11.33	20.77 ± 8.31	20.06 ± 5.25	13.8 ± 6.6
B-16 to 20	18.52 ± 5.27	15.63 ± 4.79	15.33 ± 8.96	17.99 ± 3.71	10.57 ± 6.2
B-21 to 40	22.60 ± 11.15	25.59 ± 11.12	19.43 ± 6.07	18.10 + 5.62	14.75 ± 7.1
R-1 to 15	3.87 ± 0.62	4.85 ± 1.63	4.93 ± 0.92	7.64 ± 3.00	7.93 ± 1.8
R-16 to 20	2.66 ± 1.43	5.49 ± 1.18	3.71 ± 0.73	5.48 ± 0.82	7.73 ± 1.5
R-21 to 40	2.43 ± 1.47	4.82 ± 1.18	3.77 ± 1.16	6.71 ± 1.64	7.8 ± 1.6
G-1 to 15	50.01 ± 4.36	52.51 ± 8.10	48.24 ± 8.73	41.31 ± 9.82	45.2 ± 5.7
G-16 to 20	44.04 ± 2.50	48.04 ± 3.42	47.18 ± 8.53	46.24 ± 6.89	43.49 ± 4.88
G-21 to 40	47.66 ± 7.23	46.72 ± 8.25	44.69 ± 6.31	45.60 ± 4.46	43.17 ± 6.16

*RCS—Refined common salt; CS— Common salt; DFS— Double fortified salt; IS— Iodized salt.
Note: Sample No. 1 to 15 were stored in verandah, 16 to 20 stored in outdoor condition and 21 to 40 stored in a room/godown. Y—yellow; R— red; B—blue and G—green.

under different storage conditions, while in red and blue colour packing the iodine contents were not stable. The samples in red colour packing degraded very fast, even at the initial stage the contents were almost completely degraded. These results will be correlated with different colour code of iodised/double fortified salt.