## ENTOMOLOGY

### Vector Biology

### *Anopheles culicifacies* Complex

### Bionomics and Distribution Pattern of Members

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

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

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

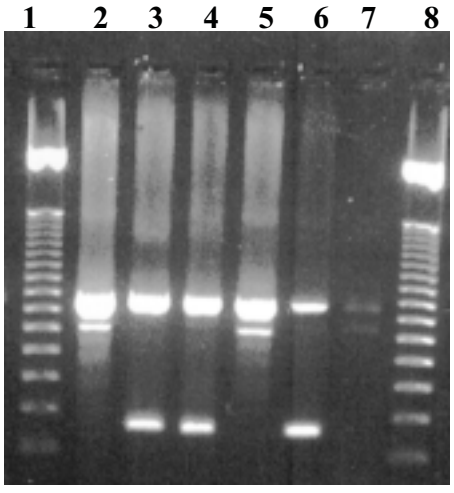



*Fig. 1:* PCR products obtained by the primers designed from D3 region of 28S rRNA electophoresed on 2% agarose gel. Lane 1 & 8 : 50 bp marker; Lane 2 : *An. culicifacies* sp. A; Lane 3 : *An. culicifacies* sp. B; Lane 4 : *An. culicifacies* sp. C; Lane 5 : *An. culicifacies* sp. D and Lane 6 : *An.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

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

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

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

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

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

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

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

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

## *Anopheles fluviatilis* Complex

### Distribution, Bionomics and Biology of Sibling Species

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

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

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

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

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

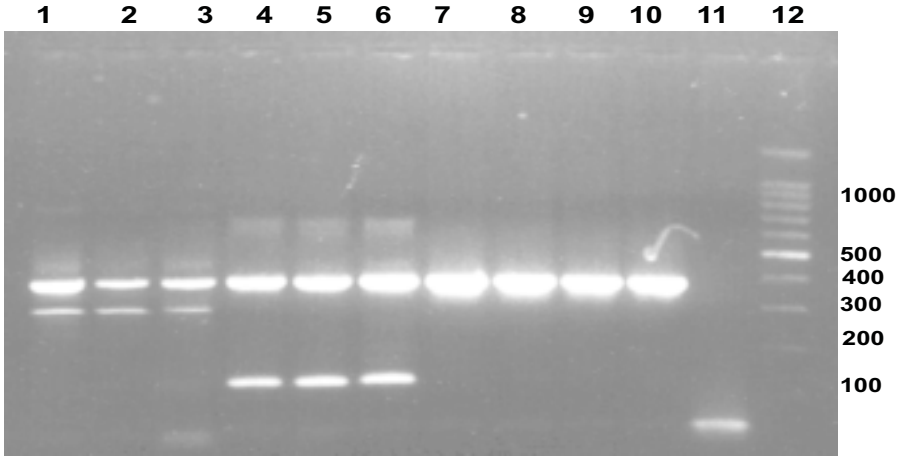


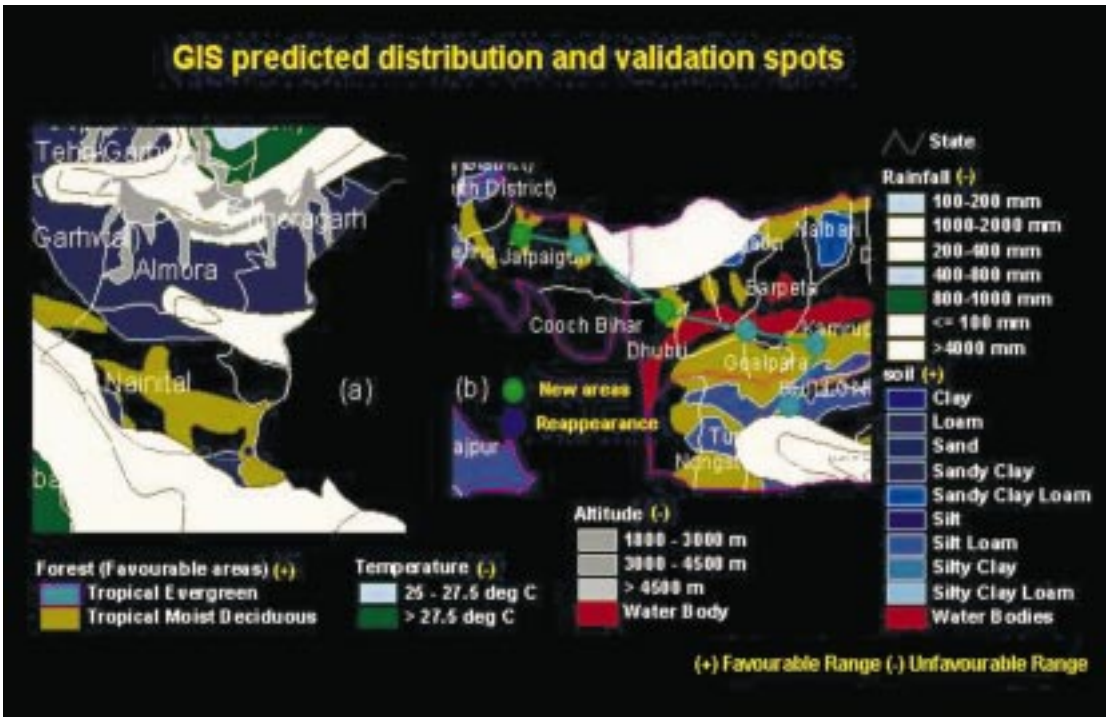

*Fig. 2:* Differentiation of members of *An. fluviatilis* species complex: PCR product as seen on 2% agarose gel containing ethidium bromide under UV illumination (Lanes 1–3, species S; lanes 4–6, species T; lanes 7–10, species U; lane 11, negative control, without DNA; and lane 12, 100 bp DNA ladder)

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

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

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

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




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

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

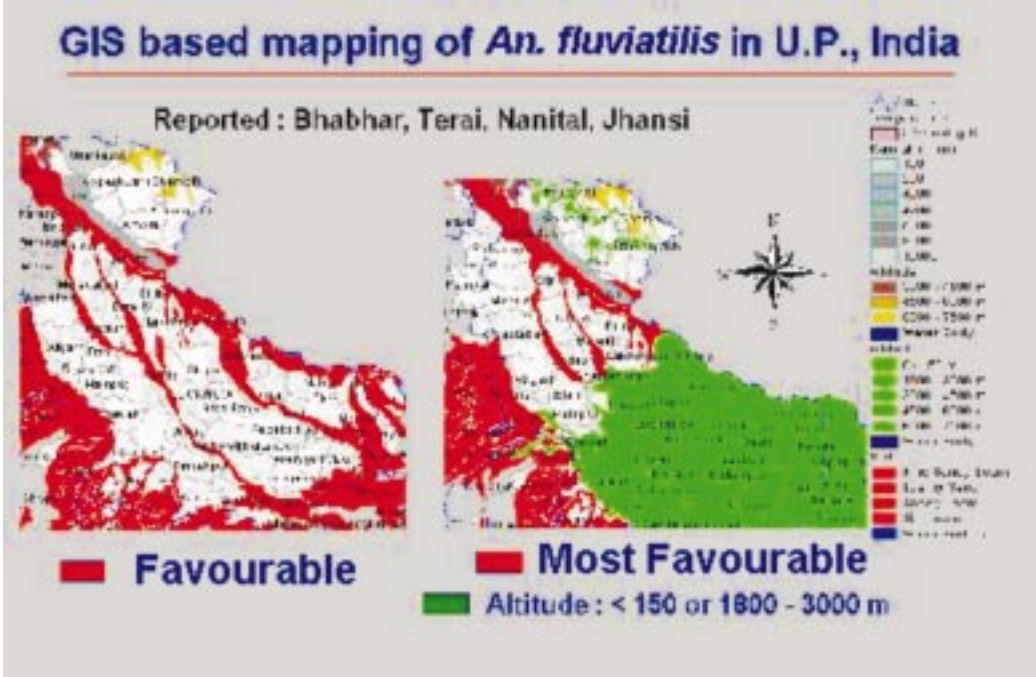



*Fig. 4:* GIS-based distribution of *An. fluviatilis* using ecological parameters such as soil, altitude, rainfall and temperature

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

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

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

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

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

## Insecticide Resistance

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

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

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

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

df—Degree of freedom.

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

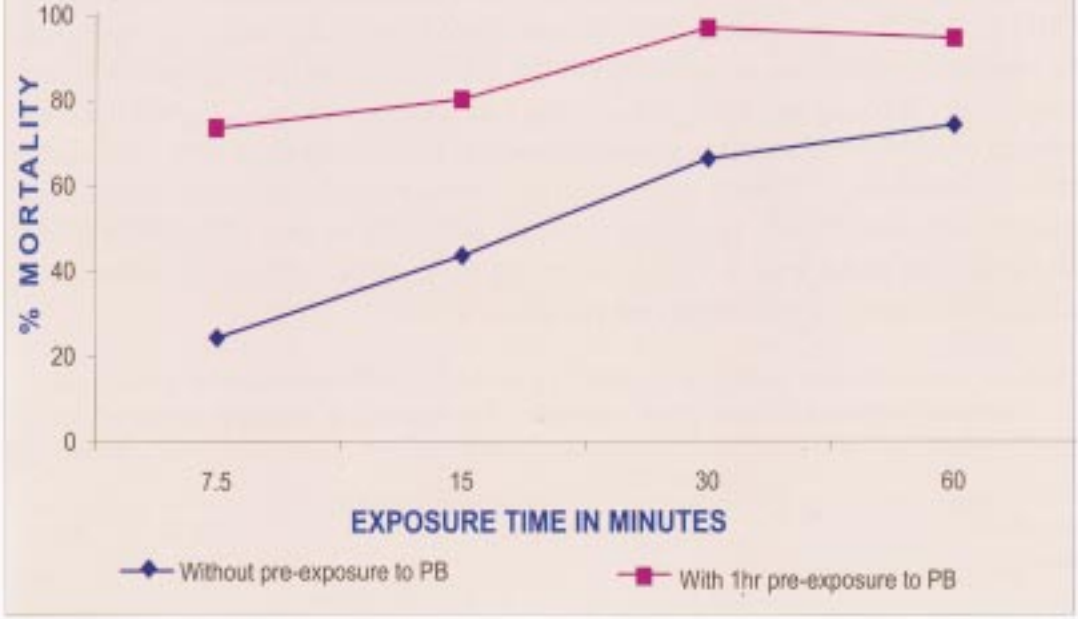
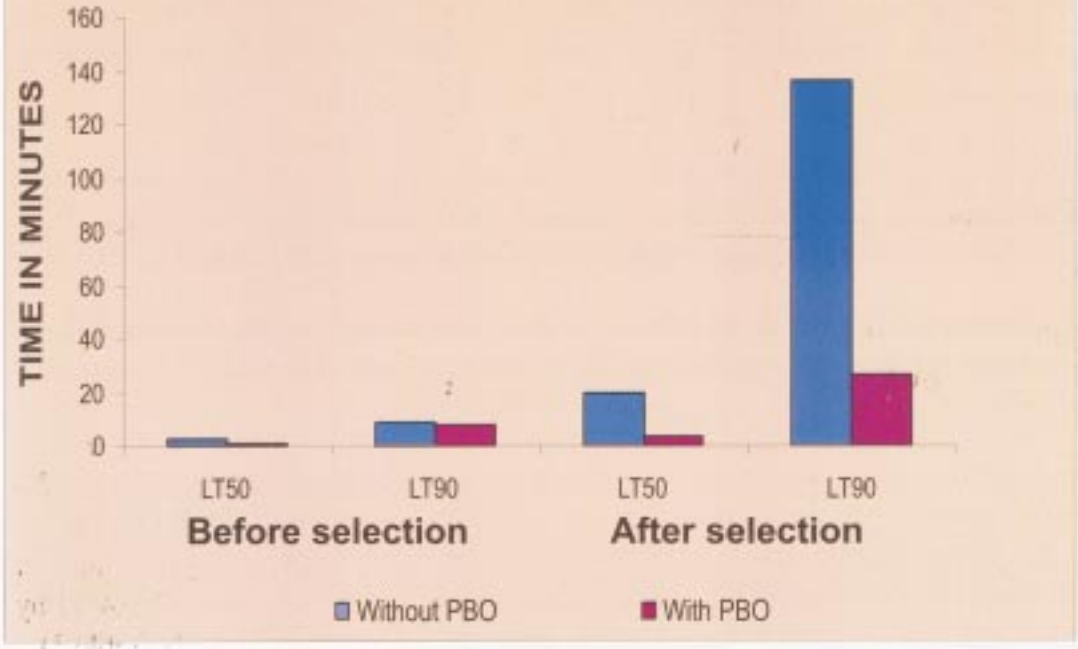



*Fig.6 :* Susceptibility of *An. culicifacies* Rameshwaram (Rmr) strain to deltamethrin (0.05%) before and after selection with deltamethrin and in the presence of PBO



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

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

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

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

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

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

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

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

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

df— Degree of freedom.

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

## Vector Control

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

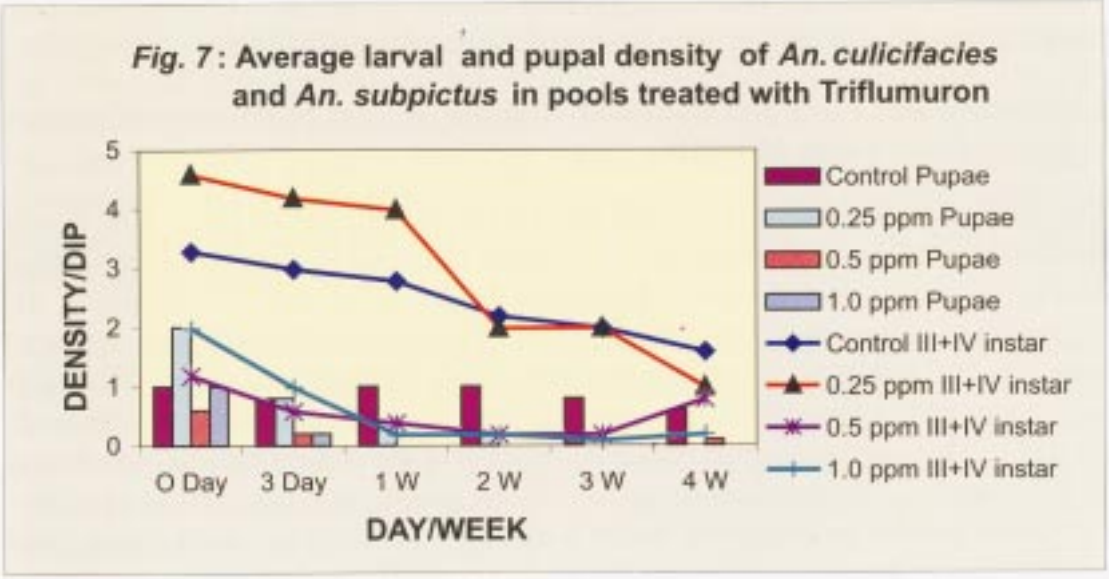



*Fig. 7*: Average larval and pupal density of *An. culicifacies* and *An. subpictus* in pools treated with Triflumuron

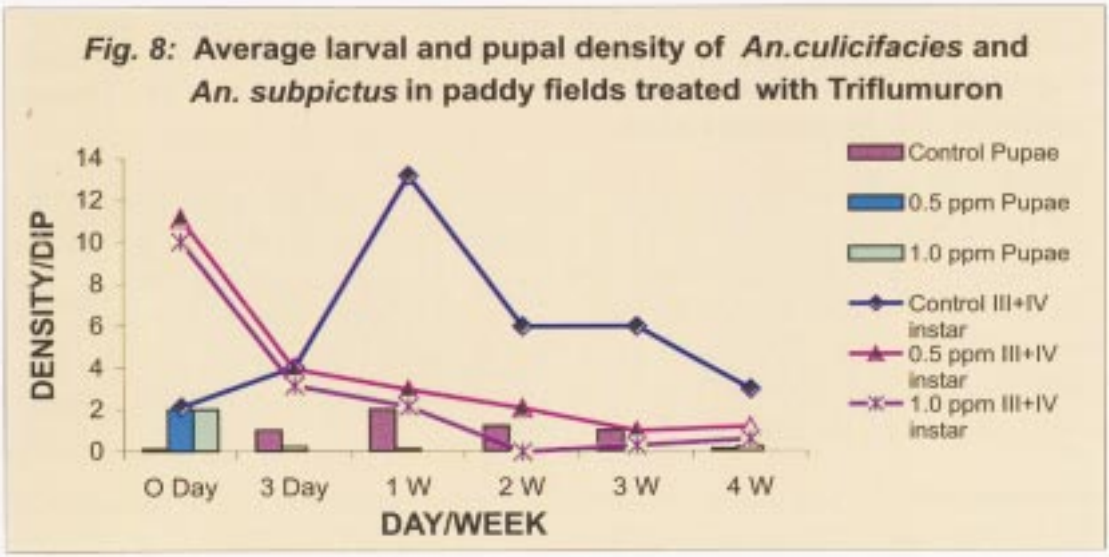
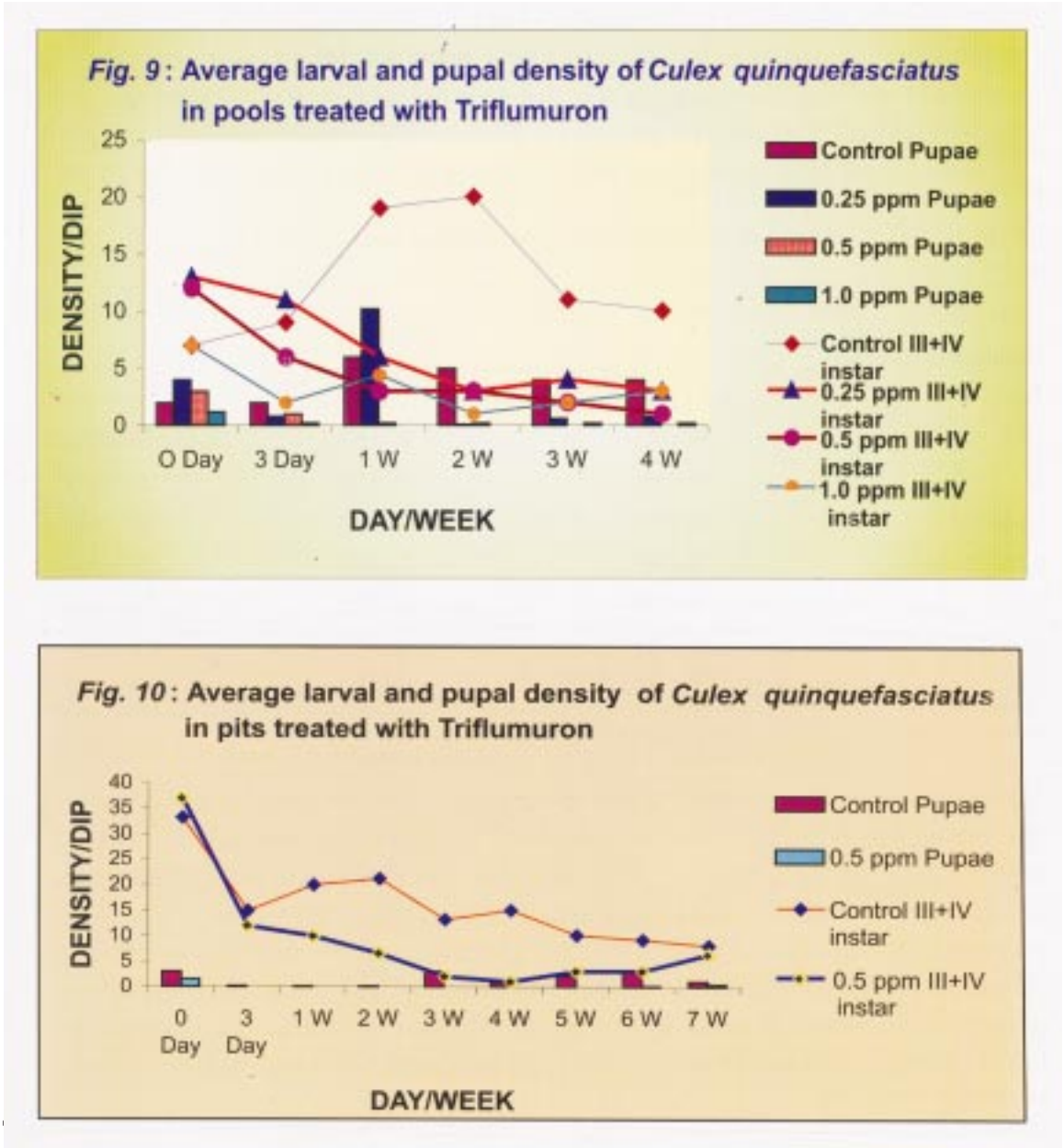



*Fig. 8:* Average larval and pupal density of *An.culicifacies* and *An. subpictus* in paddy fields treated with Triflumuron

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

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

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




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

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

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

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

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

Figures in parentheses indicate total number of larvae treated.

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