Field Evaluation of Conventional and New Insecticides

Evaluation of new insecticides has been identified as a thrust area in order to guide the NVBDCP for selection of alternative insecticides in areas with double and multiple resistance in vector species and also to suggest ways and means to prolong the useful life of conventional insecticides. In consonance with this policy a large-scale field trial was carried out in District Ghaziabad to compare the relative efficacy of DDT and HCH at single and double doses against An. culicifacies which is responsible for about 65 to 70% malaria transmission in northern plain of rural India (Ansari et al 1986). Results revealed that DDT spraying @ 1 g/m² by increasing the spray coverage from 50 to 90% has interrupted the malaria transmission in predominant area of species A which was found completely susceptible to DDT. There was also no significant difference between 1 and 2 g/m² doses of DDT spraying against this species. An. culicifacies is mainly a zoophilic species and has inherent tendency to rest in cattlesheds and houses. The study revealed that house spraying alone with >80% room coverage produced the same impact as observed in an area where both houses and cattlesheds were sprayed with similar proportion of coverage.

In addition, several new insecticides were also evaluated to tackle increasing problem of resistance and fulminating outbreaks, particularly in multi-resistant areas. Salient findings of each trial are given below.

Field Evaluation of Deltamethrin against An. culicifacies in District Ghaziabad, Uttar Pradesh

An. culicifacies, responsible for bulk of malaria transmission in rural areas, has become resistant to conventional insecticides—DDT, HCH and malathion in most parts of the country. To control double or triple resistant An. culicifacies, new insecticides, namely synthetic pyrethroids have been introduced both in the form of indoor residual spray and also for treatment of mosquito nets. The first trial of a synthetic pyrethroid by indoor residual spraying was carried out in some villages of PHC Razapur of District Ghaziabad. Deltamethrin wettable powder formulation (2.5%) was sprayed in three doses—12.5 mg/m² (3 rounds), 20 and 25 mg/m² (2 rounds each). One section in Dadri PHC located at a distance of 22 km away from this area was taken as control, where three rounds of HCH were sprayed @ 200 mg/m². Deltamethrin spraying was carried out for three years. Results revealed that spraying deltamethrin @ 25 mg/m² resulted in drastic reduction of DDT and HCH resistant An. culicifacies and other anophelines (Fig. 14) and caused interruption of malaria transmission (Fig. 15).

Fig. 14: Impact of deltamethrin spray on indoor resting densities of An. culicifacies in District Ghaziabad
Trial of New Insecticides in Collaboration with WHO Pesticide Evaluation Scheme

Phase II Evaluation of Bifenthrin

In an area in Gujarat, where *An. culicifacies*, the main vector of rural malaria has developed triple resistance to DDT, HCH and malathion, a randomised house-scale (phase II) trial of bifenthrin 10%WP was undertaken from July 1999 to March 2000. Baseline tests showed that *An. culicifacies* was 100% susceptible to bifenthrin (0.1% test papers), but only 57% to malathion (5% test papers). Entomological impact of four serial doses of bifenthrin (25, 50, 100 and 200 mg/m²) sprayed in rooms in five villages was compared with malathion (2 g/m²), and unsprayed control. In contact bioassays carried out on sprayed surfaces for 24 weeks, bifenthrin 100 and 200 mg doses caused ≥80% mortality in *An. culicifacies* till 24 weeks. The 50 mg dose caused ≥80% mortality on tin, wood and mud surfaces for 24 weeks, and on brick-walls for 16 weeks. Bifenthrin 25 mg dose produced ≥80% mortality for 24 weeks on tin, 20 weeks on mud-walls, 16 weeks on brick-walls, and eight weeks on wood surfaces. Persistence of ≥80% mortality did not differ for 25 and 50 mg doses on any surface except on wood (p <0.05). Malathion sprayed in three rounds of six weeks apart caused ≥80% mortality for 16 weeks on the brick and mud-walls, and for 20 weeks on the tin and wood surfaces. Bifenthrin 25 and 50 mg doses produced a similar impact on the densities of *An. culicifacies* and other mosquitoes but a superior one than malathion or control. Bifenthrin 25 mg dose caused least excito-repellency. Overall, efficacy of bifenthrin was superior to malathion. Considering the duration of the persistence of significant insecticidal action of bifenthrin on the most common surfaces (mud and brick-walls), least excito-repellency and a relative impact on the mosquito densities, the 25 mg dose was found to be most superior among all the four doses evaluated. The trial recommended a further village-scale (phase III) evaluation of bifenthrin 10% WP at 25 mg/m² dose (Yadav et al 2002).

Phase III Evaluation of Bifenthrin 10% WP and Deltamethrin 25% WG

In the phase III village-scale trial, bifenthrin 10% WP sprayed indoors at 25 mg/m² dose was evaluated in Gujarat state during 2000–02 to control *An. culicifacies*. An improved formulation of deltamethrin—Deltamethrin 25% wettable granules, was also evaluated by spraying indoors at 20 mg/m² during this period. Both deltamethrin and bifenthrin reduced the elements of vectorial capacity—vector densities (Figs. 16 and 17), survivorship, sporozoite rate and entomological inoculation rate significantly compared to these parameters in unsprayed control villages. A low excito-repellent action of bifenthrin caused a mass killing effect on the indoor resting population of vector mosquitoes. First round of spraying of insecticides was undertaken in mid-July 2001. Bioassays on mud-walls, which are most common surfaces, showed 100% knockdown effect up to next two months which declined markedly by the third month. The indoor resting densities of *An. culicifacies* declined significantly in sprayed villages in the month of August but increased in September though at a level much lower than in the control villages. Considering the built up of indoor resting densities, a second round of spraying was undertaken in October 2001—three months after the first round of spraying. The increasing trend of vector densities with the start of February indicated that the impact of spraying of the second round of bifenthrin lasted for three months.

Based on the detection of sporozoites in *An. culicifacies* collected in early July during the study, main period of the incidence of malaria extends from...
Field Evaluation of Conventional and New Insecticides

July to January. In a riverside village sporozoites of *P. vivax* and *P. falciparum* were also detected in *An. culicifacies* in the month of April in spring season when the vector densities were ascending. Considering the persistence of insecticidal action determined through contact bioassays on sprayed surfaces and the length of malaria transmission in this area, two rounds of spraying with bifenthrin or deltamethrin three months apart would ensure an effective reduction in malaria transmission well over six months. It would be pertinent to undertake spraying in early June and complete the first round as early as possible, preferably by the end of June to interrupt the persistent transmission of malaria during the spring. The householders did not report any adverse reaction to these insecticides. Clinical, haematological and urological examinations, and lung function and nerve conduction tests performed on volunteer spray-men showed no adverse effect on short-term relevant exposure during the trial.

**Field Evaluation of Lambda-cyhalothrin 10% Capsule Suspension**

A village-scale field trial was carried out to evaluate a synthetic pyrethroid insecticide, Lambda-cyhalothrin 10 CS (10% capsule suspension formulation) as an indoor residual spray in District Tumkur in Karnataka state and in Districts
Dharmapuri and Ramanathapuram in Tamil Nadu during 2005–07. Evaluation was done to assess the entomological and epidemiological impact of the insecticide on malaria transmission. Results were compared with the impact of the routinely used insecticides in indoor residual sprays by the National Vector Borne Disease Control Programme (NVBDCP). The evaluation was carried out essentially following the ‘Protocols for uniform evaluation of insecticides for use in vector control’ jointly published by National Institute of Malaria Research, Delhi and Vector Control Research Centre, Puducherry. The district-wise observations are as follows:

**District Tumkur (Karnataka)**

In this district villages of PHC Taverkere were selected for the studies. Lambda-cyhalothrin 10 CS was sprayed in four villages (experimental) @ 25 mg/m² comprising of a population of 3098 while malathion was sprayed in three villages (control) comprising of 2820 population. The villages had similar ecotype and *An. culicifacies* was the principal vector of malaria in this region. In insecticide susceptibility tests *An. culicifacies* registered 82 and 97% mortality to malathion in experimental and control areas while against lambda-cyhalothrin 82 and 93% respectively. Insecticide spray was done following the insecticide spray schedule followed by NVBDCP. Two rounds of lambda-cyhalothrin 10 CS was sprayed in experimental villages and the room coverage in first round (October 2005) and second round (February 2006) was 67.4 and 69%, respectively. Two rounds of malathion (first round September 2005 and second round February 2006) were sprayed in control villages with respective room coverage of 59 and 55.7%. The pre-spray mortalities in cone bioassays in experimental and control villages in *An. culicifacies* on wall surfaces were 29 and 33% respectively. After first round spray the species registered 100% mortality to lambda-cyhalothrin 10 CS sprayed surfaces in experimental areas while in control villages on malathion sprayed surfaces it was 86%. The mortality in cone bioassays on lambda-cyhalothrin sprayed surfaces was >80% up to three months after spray while in the control area on malathion sprayed surfaces it was >80% only in the first month and decreased by second month (the residual efficacy of malathion is 6–8 weeks). The man hour densities (MHD) of *An. culicifacies* in the pre-spray period in experimental and control areas was 16.4 and 24.3 respectively. The MHD reduced to 0.7 (range 0.14–1.7) after first round in the experimental area while it was 8.0 (range 1.4–40) in the control area. In post-spray period of second round the MHD in experimental area was in the range of 0–0.5 while in the control area it was 1.0–2.5. Lambda-cyhalothrin 10 CS has shown relatively higher efficacy. Pre-spray densities in total catch collection in the experimental area in cattlesheds and human dwellings were 6 and 18 respectively while in the control area it was 51 and 14 respectively. In the immediate post-spray periods the densities decreased in both the experimental and control areas. However, the impact of lambda-cyhalothrin 10 CS IRS was better than malathion IRS. The post-spray densities in experimental area in human dwellings was in the range of 1–4 (post-spray first round—1–4 and post-spray second round it was >1). The densities in post-spray period in control areas were in the range of 2–22 (post-spray first round—10–22 and post-spray second round it was >3).

The parity rates during pre-spray period were 69 and 75 respectively in the experimental and control areas and decreased to 39 and 43 respectively after the spray. In both the experimental and control areas malaria incidence reduced after spray in comparison to pre-spray. Human safety of lambda-cyhalothrin 10 CS was evaluated in human volunteers (spraymen and inhabitants) in experimental area on general health, six biochemical parameters and routine and biochemical analysis of urine and differential counts of blood. No significant changes were observed in profiles in the pre- and post-spray periods. Inhabitants have expressed satisfaction about the benefits of spray in their villages and did not report any adverse effect during or after the spray operation.

**District Dharmapuri, Tamil Nadu**

In District Dharmapuri, two villages Hoggenakal and Ootamalai PHC Nagadasampatti, comprising of a population of 2810, were selected for evaluation of lambda-cyhalothrin 10 CS (experimental area). Five villages, namely Balrampatti, Seriapatti, Chinnappankottai, Pooniyantottai and Sekinatham were selected as control villages where deltamethrin 2.5% WP was sprayed as a comparative insecticide. *An. culicifacies* is the vector of malaria in this region. First round of spray in experimental villages with lambda-cyhalothrin 10 CS was carried out in February 2006 with house coverage of 66.3%. Second round spray in experimental villages was carried out in July 2006 with 72% house coverage. In control villages deltamethrin 2.5 WP was sprayed in first round (March 2006) with 51.9% house coverage which in second round (August 2006) increased to 59.7%. Pre-spray MHD of vectors in experimental villages was 12 and that of in control villages it was 8. In the post-lambda-cyhalothrin spray period in experimental villages the density was >1. While in control villages sprayed with deltamethrin, the MHD was >1 in the post-spray period after first round and was in the range of 2–4 in post-spray period after second round. The impact of lambda-cyhalothrin 10 CS was found superior than deltamethrin. Per-structure density in total catch collection of *An. culicifacies* was respectively 9 and 8 in experimental and control villages during pre-spray. After the first round spray the per-structure density in the experimental area was 0–2 and 4–7 in...
the control area. After the second round spray it ranged from 4–6 in the experimental villages while it ranged from 2–13 in the control villages. Similar impact was found on total anophelines and Culex species populations. The impact was significant (p<0.05) indicating superior efficacy of lambda-cyhalothrin 10 CS being a slow release formulation. Persistence studies indicated similar efficacy of the two insecticides in comparison. The residual activity (80% mortality rate of mosquitoes) remained for three months after the first and second rounds of spray in the experimental area and up to three months and two months respectively in control area.

Parity rates of An. culicifacies decreased from 76 in pre-spray period to 44 in post-spray period in the experimental area and from 68 to 42 in the control area indicating equal effectiveness of the insecticides (p>0.05). Active and passive surveillance studies indicated prevalence of both P. vivax and P. falciparum with increased prevalence of P. vivax in the experimental area. In post-monsoon months 18 P. falciparum cases were reported in the control area as against two cases in experimental areas indicating continued transmission in the control area. Though the number of malaria cases reported in the experimental village was slightly higher than the control village, statistically no significant difference was observed between the two villages (χ² = 1.56; p>0.05). Similarly, in mass blood surveys P. falciparum cases were found prevalent in control villages. It may be stated that the villages in both areas were receiving deltamethrin spray and being an effective insecticide kept the transmission under control. However, lambda-cyhalothrin CS has shown better effect in curtailing the malaria transmission. No adverse effects were reported by inhabitants or spraymen during the spray and overwhelming response was received from the inhabitants for IRS.

**District Ramanathapuram (Tamil Nadu)**

In District Ramanathapuram, villages of PHC Pamban in Rameshwaram Island were selected for the studies. Five villages, namely Mundal, Thoppukadu, Chinnapalam, Kundakal and Tharavai Thopu comprising a population of 2827 were selected as experimental villages and village Akkalamaddam and hamlets comprising of a population of 2626 were selected as control villages. An. culicifacies is the vector of malaria in these villages. Spray was carried out as per the NVBDCP schedule of indoor residual spray. Both experimental and control areas received different formulations, CS and WP of lambda-cyhalothrin. Three rounds of lambda-cyhalothrin 10 CS spray (July 2006, November 2006 and March 2007) was carried out in experimental villages with respective per cent house spray coverage of 98.6, 98.2 and 98.6%. In control villages, three rounds of lambda-cyhalothrin 10 WP IRS was carried out simultaneously with 95.5, 90.8 and 81.4% coverage in first, second and third rounds respectively.

The MHD of An. culicifacies remained low in experimental areas throughout the study period (0–2) while in the control area it reached up to 4.5. Total catch assessment in structures in post-spray period indicated per structure density of An. culicifacies as 0–4 except in March 2007, and of anophelines remained in the range of 1–5 except in November 2006. While in control area it was below 12 except in October and November 2006. Similar impact was seen on Culex species density. This data on indoor densities indicated better impact of lambda-cyhalothrin 10 CS probably owing to the slow release characteristic of the CS formulation over the WP formulation. A significant decrease in parity rates (p<0.05) was observed. Pre-spray parity rate of the vector in the experimental area was 62 and decreased to 22 in post-spray period. Similarly, in the control area it decreased from 58 to 24. The malaria incidence in the experimental and control villages was very low throughout the study period. The positives in experimental area was 31 (20 P. vivax + 11 P. falciparum) while in control area it was 61 (45 P. vivax + 16 P. falciparum) indicating a better interruption of transmission in the experimental villages. Mass blood surveys did not show any difference between the control and experimental villages indicating equal effectiveness of insecticides. Inhabitants and spraymen did not report any adverse effects due to spray operation. There was an overwhelming response from the inhabitants for the spray.

Multicentric field trials at three sites has established that lambda-cyhalothrin 10 CS formulation was found relatively more effective than malathion 25% WP, deltamethrin 2.5 WP and lambda-cyhalothrin 10 WP in some important evaluation parameters like indoor resting mosquitoes, parity rates of vector mosquitoes, increased persistence, etc.

**Evaluation of Chlorfenapyr (Phase I) (Pyrrole Insecticide) against Susceptible and Resistant Strains of Mosquito Species**

Phase 1 evaluation of Chlorfenapyr, of pyrrole group of insecticides, was carried out following WHO guidelines and common protocols published jointly by NIMR, Delhi and VCRC, Puducherry. Chlorfenapyr is a pro-insecticide which is converted by oxidases in animals to an active form. This insecticide is a mitochondrial electron transport inhibitor (METI) whose mode of action is to disrupt the conversion of ADP to ATP (Oxidative phosphorylation) in mitochondrion. Because chlorfenapyr’s mode of action is novel, it is unlikely to show cross resistance to neurotoxic insecticides. It has low mammalian toxicity (acute oral rat—2582 mg/kg BW) and classified as WHO toxicological class III and placed in the category ‘slightly hazardous’ to humans. The insecticide has shown good efficacy against three species of mosquitoes, An. culicifacies, An. stephensi and Cx. quinquefasciatus. The efficacy against Ae. aegypti was erratic and hence was not included in
the study. Adult bioassays with impregnated paper were carried out using different concentrations and exposure times. The results showed that exposure to impregnated papers for 2 h and 48 h holding period was suitable for susceptibility tests. Susceptibility was determined by exposing the mosquitoes to impregnated papers in a range of concentrations from 0.25 to 5% for 2 h exposure and holding period of 48 h. The dose-mortality response data were subjected to log-probit regression analysis and LD$_{50}$ and LD$_{99}$ value were calculated. LD$_{50}$ values for *An. culicifacies* species A was 0.411%, species C was 0.676%, *Cx. quinquefasciatus* was 0.370% and *An. stephensi* was 0.437%, while LD$_{99}$ were respectively 2, 2.39, 2.23 and 2.13%. Thus, LD$_{99}$ was in the range of 2 to 2.4% for different species. As per the standard criteria double the LD$_{99}$ was chosen as the diagnostic dose for assessment of susceptibility in the field mosquitoes. Accordingly, 5% impregnated paper with 2 h exposure and 48 h holding is suggested for assessing the susceptibility of mosquitoes in field. Persistence studies were carried out on five different fabricated surfaces, namely mud, mud + lime, cement, cement + distemper and wood. These surfaces were sprayed with Chlorfenapyr 10% SC was sprayed in a range of 12.5 to 800 mg/m$^2$ following NVBDCP norms. Efficacy of insecticide on the sprayed surfaces was assessed by cone bioassays with 30 min exposure and 24 h holding period and the results indicated a dose of 400 mg/m$^2$ to be appropriate dose for effectiveness. Chlorfenapyr @ 400 mg/m$^2$ was found to be effective up to 28 weeks against *An. culicifacies* and up to 34 weeks with *An. stephensi*. While against *Cx. quinquefasciatus* two surfaces namely mud + lime and wood have shown consistent results up to 34 weeks and with other surfaces persistence of effectiveness was variable. Studies to assess cross resistance with 5% chlorfenapyr impregnated papers for 120 min with laboratory selected permethrin resistant *Cx. quinquefasciatus* (74%) has shown 100% mortality at the end of 48 h while lambdacyhalothrin resistant *Cx. quinquefasciatus* (78%) has shown 100% mortality within 24 h. In exposures with 2% chlorfenapyr for 60 min in *An. culicifacies* field strain (Surat) which was double resistant to DDT (80%) and malathion (43%) registered 100% mortality within 24 h. No side-effects such as eye irritation, skin irritation, etc. were reported during and after spray by the applicators and investigating staff.

**Multicentric Study on the Susceptibility of Culex quinquefasciatus Larvae to Fenthion in Urban Areas**

Application of Fenthion (100% EO) @ 5 cc in 10 litres in breeding habitats of *Cx. quinquefasciatus* @ 20 cc/m$^2$ was carried out in urban areas of Delhi, Bengaluru and Nadiad from September to November 2006, to evaluate the efficacy in field conditions.

The results revealed that the spraying of Fenthion produced a very low impact on larval densities in general. The effect lasted only up to three days in Delhi and Nadiad and till seven days of post-spray in Bengaluru with 71.98% reduction after first round and no reduction after third round of spray. The gradual rounds of spray resulted in further reduction in larval densities. On Day 21 of post-spray, the larval density was more than Day 0, indicating no impact of larvicide in Delhi, Nadiad and Bengaluru.

The results of the larval susceptibility tests on *Cx. quinquefasciatus* indicated that the corrected percent mortality in larvae at the dose of 0.05 ppm was 18.7, 53.4 and 30.5 in Nadiad, Delhi and Bengaluru respectively which revealed that *Cx. quinquefasciatus* has developed resistance to the insecticide in all the study areas.

**Trials of Insecticide-treated Mosquito Nets and Curtains**

Nets or curtains treated with various formulations of pyrethroids such as deltamethrin, cyfluthrin, lambda-cyhalothrin and bifenthrin have been evaluated in laboratory and through field trials. The results are given specifically in this publication.