

# Insecticide Resistance: Monitoring and Biochemical Mechanisms

Indoor residual spraying (IRS) with insecticides such as DDT, HCH and malathion has been the main strategy to control malaria vectors in India under the National Anti Malaria Programme (Now National Vector Borne Disease Control Programme). Continuous usage of insecticides under National Anti Malaria Programme has resulted in the development of resistance to different insecticides in major malaria vector species.

## Resistance Monitoring

Monitoring of insecticide resistance in malaria vectors is an important activity performed along with other entomological studies. Resistance monitoring will be of use in formulating suitable situation-specific insecticide-based vector control strategies and most importantly for the management of insecticide resistance in malaria vectors.

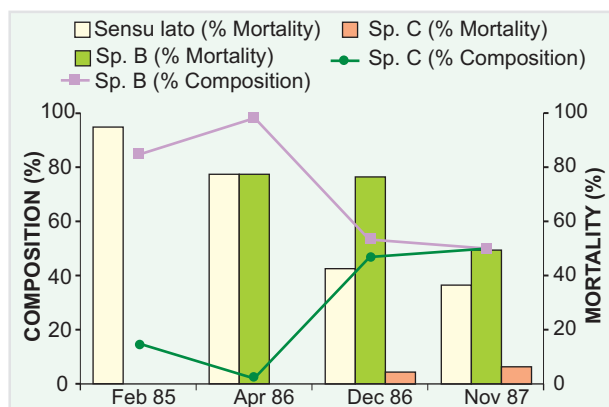
Results of insecticide susceptibility tests carried out by NIMR using WHO diagnostic dose bioassays or by dose-response bioassays, during the past decades have shown that *An. culicifacies*, the major vector of malaria in most of the rural plain areas of India has developed varying degree of resistance to DDT and HCH in different parts of the country (Ansari *et al* 1986, 1988, 1990; Dhiman *et al* 2001; Sharma 1996; Sharma *et al* 1982, 1986; Shukla *et al* 1995; Singh and Sharma 1989; Singh *et al* 1989; Srivastava *et al* 1995; Subbarao *et al* 1984, 1988). It has also become resistant to malathion in most parts of Maharashtra and

Gujarat and also in various districts of Andhra Pradesh, Madhya Pradesh, Haryana, Punjab, Karnataka and Tamil Nadu (Ansari *et al* 1988, 1990; Batra *et al* 1999; Raghavendra *et al* 1992, 1997, 1998). In Andhra Pradesh, our studies have shown that *An. culicifacies* developed resistance to malathion in the absence of IRS for malaria control owing to selection by pesticides used to protect cash crops like chilli, cotton and tobacco (Raghavendra *et al* 1991). Recently in District Chhindwara, Madhya Pradesh, a focus of malathion resistance was found in *An. culicifacies*. As in Andhra Pradesh the species has developed resistance in the absence of indoor spraying of malathion in public health and probably due to the use of pesticides in agriculture and forestry. During the surveys in Gujarat a focus of synthetic pyrethroid-resistant *An. culicifacies* has been found in some PHC areas of District Surat (Singh *et al* 2002). Monitoring studies in Rameswaram Island in Tamil Nadu state have also shown reduced susceptibility to deltamethrin in *An. culicifacies* (Mittal *et al* 2002).

We have also studied resistance status in sibling species of *An. culicifacies*. These studies have shown differential response to DDT and malathion in sympatric species A and B prevalent in Uttar Pradesh and Haryana respectively. Species A was found more susceptible to DDT and malathion than species B (Raghavendra *et al* 1992; Subbarao *et al* 1988). On the contrary, in Gujarat, Maharashtra and Andhra Pradesh, where species B and C are sympatric, species C became resistant to malathion within 2–3 years, while species B was still half as resistant as species C (Fig. 45) (Raghavendra *et al* 1991, 1998).

Susceptibility tests carried out at NIMR and elsewhere have also shown that *An. stephensi*, a predominant vector of malaria in urban areas in India has developed wide-spread resistance to DDT in different parts of the country (Sharma 1996). Resistance to malathion has also been reported from Maharashtra, Gujarat, Karnataka and Tamil Nadu. Further, studies carried out at NIMR have shown development of resistance to malathion in Haryana and Goa (Subbarao *et al* 1984 ; Thavaselvam *et al* 1993).

*An. fluviatilis*, another major vector was resistant to DDT in *terai* region of Uttar Pradesh (Sharma *et al* 1999), while it was found susceptible to DDT in Orissa (Chand and Yadav 1991). Susceptibility tests of *An. minimus* in District Kamrup in Assam and *An.*



**Fig. 45:** Differential development of malathion resistance in sympatric *Anopheles culicifacies* sibling species B and C under the selection pressure of agriculture pesticide sprays on cash crop cultivation in District Mahabubnagar, Andhra Pradesh

*sundaicus* in Car Nicobar Islands with DDT and other insecticides have shown complete susceptibility. Field studies on insecticide resistance were carried out in November 2005 and November 2006 in villages of Ukalda PHC area (District Surat, Gujarat), which were under regular IRS till 2000. Malathion replaced with DDT in IRS in 1970s, and later pyrethroids were introduced in mid 1980s. Our earlier studies in 1987, 1992 and 2002 have indicated development of resistance to DDT, malathion and also to deltamethrin in 2002. Insecticide resistance status to different insecticides was determined using WHO bioassays, resistance mechanisms by microplate biochemical assays and species composition by ASPCR assays.

In 2005, this species was reported resistant to DDT (60%) and malathion (34%) but was susceptible to other organophosphates (fenitrothion 97%), carbamates (propoxur 95%; bendiocarb 100%) and pyrethroids (deltamethrin 98%; lambda-cyhalothrin 97%; cyfluthrin 96.8% and permethrin 99.2%). While in 2006, it was relatively more susceptible to DDT (80%) and malathion (43%) but has registered similar susceptibility to organophosphates, carbamates and pyrethroids. On the contrary, it was 31–40% resistant to deltamethrin in 2002 and to malathion it was 84% in 1987. Though DDT was withdrawn from regular sprays in 1970s, resistance in populations is maintained and may be due to the presence of DDT on walls which imparted continued selection or might have genetically weighed down the negative selection of DDT by removing the negative fitness costs of the resistant genes.

Synergistic bioassay with triphenyl phosphate (TPP), a specific inhibitor of carboxyl esterase indicated complete synergism with 10% TPP impregnated paper indicating involvement of carboxylesterase as major mechanism for conferring malathion resistance in this species as observed earlier. Field-collected female *An. culicifacies* s.l. mosquitoes and the dead and alive mosquitoes in the insecticide exposures in WHO susceptibility tests were stored in isopropanol and later identified to sibling species using ASPCR assays (Goswami *et al* 2006). Major sympatricity of species B (59.4%) and E (39.2%) was found while of species C it was only 1.2%. Prevalence of species E from this area is being reported for the first time. Identification of dead and alive mosquitoes in the insecticide bioassays to sibling species indicated differential susceptibilities in sibling species to DDT and malathion. To DDT, species B was 31.5% susceptible while to malathion it was 64% and species E was 56.2 and 75% susceptible to these insecticides respectively. Thus, species B registered relatively more resistance to DDT and malathion.

In November 2006, species composition registered an increase in species C and was, species B (55%), E (32%) and species C (13%). Susceptibilities to DDT and malathion respectively in species B were 24 and 54%; species E 14 and 52% and species C, 50

and 48%. Withdrawal of IRS for 5–6 years has shown reversal of resistance especially to pyrethroids. This could be a strategy for the management of resistance in malaria vectors and if needed another insecticide with different mode of action can be used and reduce the continued selection pressure of insecticides in use.

### Diagnostic concentrations for alpha-cypermethrin and bifenthrin for malaria and dengue vectors

Six graded doses of alpha-cypermethrin (0.001 to 0.05%) and bifenthrin (0.01 to 0.5%) were tested using standard WHO protocols. Three-day old sugar-fed mosquitoes were used for the assays. *An. culicifacies* registered 98% mortality against alpha-cypermethrin at 0.0025% concentration and against bifenthrin at 0.1% while *An. stephensi* registered 94% mortality against 0.05% alpha-cypermethrin and 99% against 0.25% bifenthrin. Likewise, *Ae. aegypti* registered only 91% mortality against 0.05% alpha-cypermethrin and 99% against 0.25% bifenthrin. Results were communicated to WHO for determination of diagnostic doses against these insecticides taking into view the results from other participating Institutes.

### Biochemical Mechanisms of Resistance

Detection of biochemical mechanisms responsible for the development of resistance indicate the possible early onset of resistance, and from the type of mechanisms detected, one could predict the cross- and multiple-resistance patterns, the resistant insect would exhibit.

### Organophosphate Resistance

Microplate biochemical assays carried out on field-collected malathion resistant *An. culicifacies* species A, B and C indicated the non-involvement of elevated levels of non-specific esterases (Fig. 46) and insensitive acetyl cholinesterase (Fig. 47) which are responsible for organophosphate resistance. Bioassays with synergist triphenyl phosphate (a specific carboxylesterase inhibitor) have indicated the involvement of carboxylesterase as the major

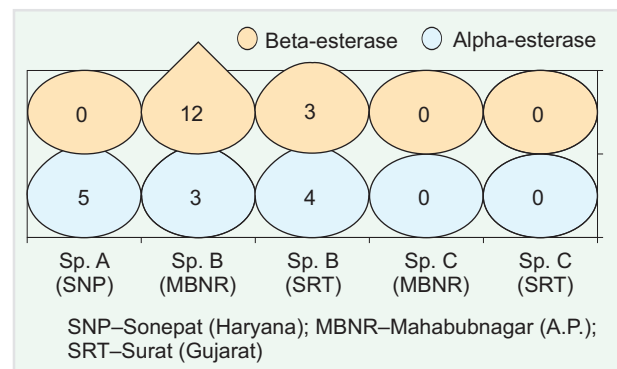
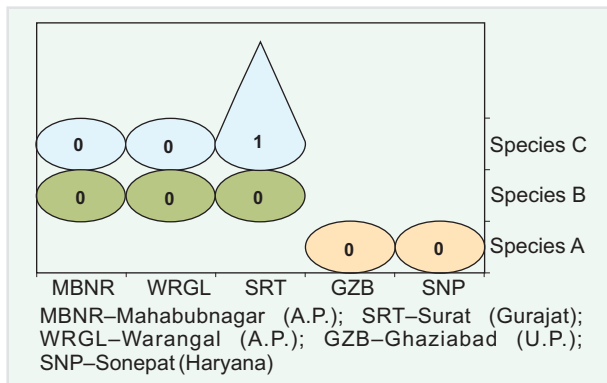


Fig. 46: Proportions of the *An. culicifacies* sibling species populations showing activities below the observed threshold values (as observed for the susceptible strains) of esterases



**Fig. 47: Proportions of sympatric sibling species populations showing uninhibited activity above 100% activity of acetyl cholinesterases**

mechanism of malathion resistance in these species (Fig. 48) (Raghavendra *et al* 1998).

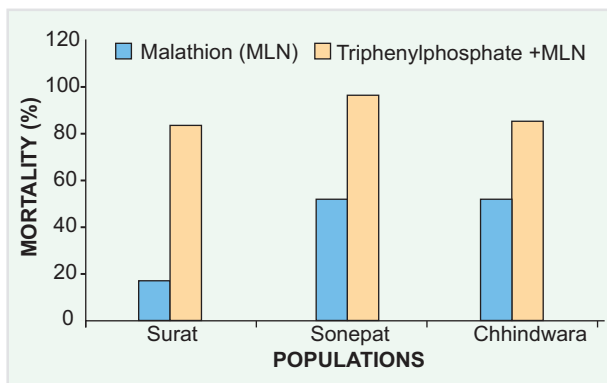
Similar profile of resistance mechanism for organophosphate insecticides was observed in *An. stephensi*. Further, study on malathion metabolism in malathion resistant and susceptible strains of *An. culicifacies* revealed the metabolism of malathion to mono- and di-carboxylic acids in malathion resistant strain which confirmed the involvement of malathion carboxylesterase in malathion resistance in *An. culicifacies*. The selection of this mechanism results in narrow spectrum resistance and in such a situation malathion can be replaced with other organophosphorous and carbamate insecticides to control malathion resistant vector species.

### Pyrethroid Resistance

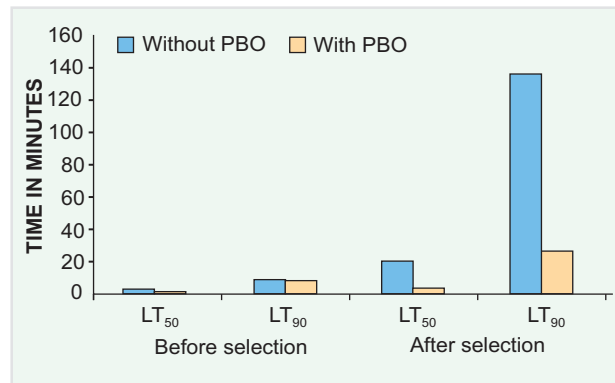
Synergistic studies on deltamethrin resistant strain of *An. culicifacies* s.l. with piperonyl butoxide (PBO—a monooxygenase inhibitor) indicated the involvement of monooxygenases in conferring deltamethrin resistance (Fig. 49).

### DDT Resistance

Microplate assays on DDT resistant *An. culicifacies* species B and *An. culicifacies* s.l. strain from Rameswaram and DDT susceptible species A, for Glutathione-s-transferase (GST) activity, revealed



**Fig. 48: Results of synergistic bioassays to determine the malathion resistance in mosquitoes**

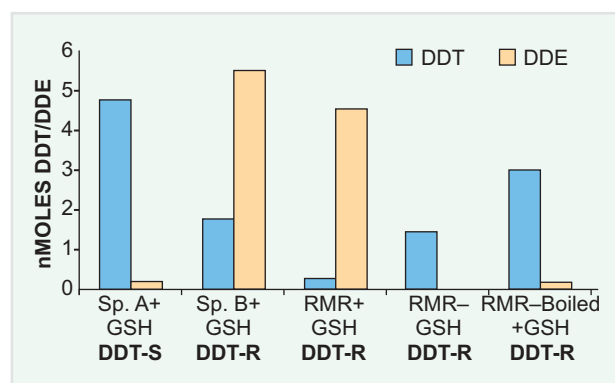


**Fig. 49: Susceptibility of *An. culicifacies* Rameswaram (Rmr) strain to deltamethrin (0.05%) before and after laboratory selection with deltamethrin and in the presence of PBO**

a significantly higher titres of GST activity in DDT resistant strains than in DDT susceptible strain, indicating the involvement of GSTs in conferring DDT resistance in *An. culicifacies*. Further studies on DDT metabolism using HPLC showed enhanced metabolism of p-p' DDT to p-p' DDE in the DDT resistant strains in the presence of reduced Glutathione, which confirmed the involvement of GSTs in DDT resistance in *An. culicifacies* (Fig. 50).

This information on biochemical resistance mechanism(s) in conjunction with results of insecticide bioassays will be of use to develop suitable insecticide spray strategies for insecticide resistance management in disease vectors.

Surveys were carried out in November 2001, March 2002, March 2003 and October 2003 in two groups of villages (3 and 4 villages) in District Chhindwara for detection and characterisation of organophosphate-resistance in *An. culicifacies* sibling species in Madhya Pradesh. First group of villages was under regular spray of DDT in public health while the second group was on the Madhya Pradesh-Maharashtra state border about 100 km from the first group and not under IRS since last ten years. Malathion was never sprayed in the public health sprays in this district. Standard WHO methods were



**Fig. 50: Relative ratio of DDT metabolised to DDE *in vitro* in *An. culicifacies* with different susceptibility status to DDT and in the presence or absence of reduced glutathione (GSH)**

used for assessing the resistance, microplate assays for the detection of resistance mechanisms and PCR assays for identifying the sibling species.

*An. culicifacies*, the major vector of malaria was resistant to DDT (~20–30%) to malathion it was ~52.6% resistant in susceptibility tests in both the areas ( $LT_{50}$  62.44 min and  $LT_{90}$  250.35 min). Pesticides of different groups including organophosphates were in regular use in this district in agriculture and forestry. The species was found completely susceptible to carbamates—propoxur ( $LT_{90}$  22 min) and bendiocarb ( $LT_{90}$  36.54 min) and to synthetic pyrethroid—deltamethrin ( $LT_{90}$  19.44 min) indicating narrow spectrum resistance, *i.e.* to malathion alone.

Synergistic bioassays with carboxylesterase inhibitor TPP (10, 15 and 20%) and mixed function oxidase inhibitor, PBO (10, 15 and 20%) revealed synergism with TPP indicating involvement of carboxylesterase for conferring malathion resistance which was also confirmed in biochemical assays. Species B and C were sympatric comprising, 73 and 27% ( $n = 138$ ) respectively. In bioassays these were respectively 68 and 13.5% susceptible to malathion ( $p > 0.001$ ). Thus, Species C was relatively more resistant to malathion than species B as was observed in our earlier studies in Andhra Pradesh where similar prevalence of sibling species and selection was found, *i.e.* by agriculture pesticides.

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