

Insecticide Resistance: Monitoring and Biochemical Mechanisms

Indoor residual spraying 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. Continuous usage of insecticides under National Anti Malaria Programme has resulted in the development of resistance to different insecticides in the 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 for formulating suitable situation specific insecticide based vector control strategies and most importantly for management of insecticide resistance in malaria vectors.

Results of insecticide susceptibility tests carried out by MRC using WHO diagnostic dose bioassays or by dose-response bioassays, during the past two decades have shown that *An. culicifacies*, the major vector of malaria in most of 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 *et al.*, 1982, 1986; Sharma, 1996; 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 indoor residual spray 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 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 in Gujarat state (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

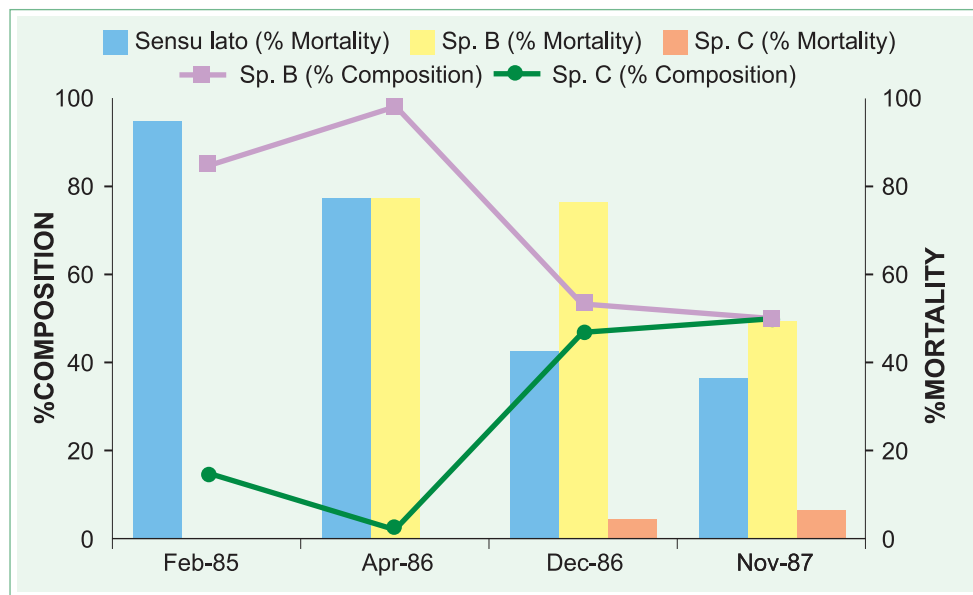


Fig. 43: **Differential development of malathion resistance in sympatric sibling species B and C under the selection pressure of agriculture pesticide sprays on cash crop cultivation in District Mahabubnagar, Andhra Pradesh**

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. 43) (Raghavendra *et al.*, 1991, 1998).

Susceptibility tests carried out at MRC 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 also has been reported from Maharashtra, Gujarat, Karnataka and Tamil Nadu. Further studies carried out at MRC 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 U.P. (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. sundaicus* in Car Nicobar Islands with DDT and other insecticides have shown complete susceptibility.

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.

Organo-phosphate Resistance

Microplate biochemical assays carried out on field collected malathion resistant *An. culicifacies* species A, B and C indicated the noninvolvement of elevated levels of nonspecific esterases (Fig. 44) and insensitive acetylcholinesterase (Fig. 45) which are responsible for organophosphate resistance. Bioassays with synergist tri-phenyl phosphate (a specific carboxyl esterase inhibitor) have indicated the involvement of carboxyl esterase as the major mechanism of malathion resistance in these species (Fig. 46) (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

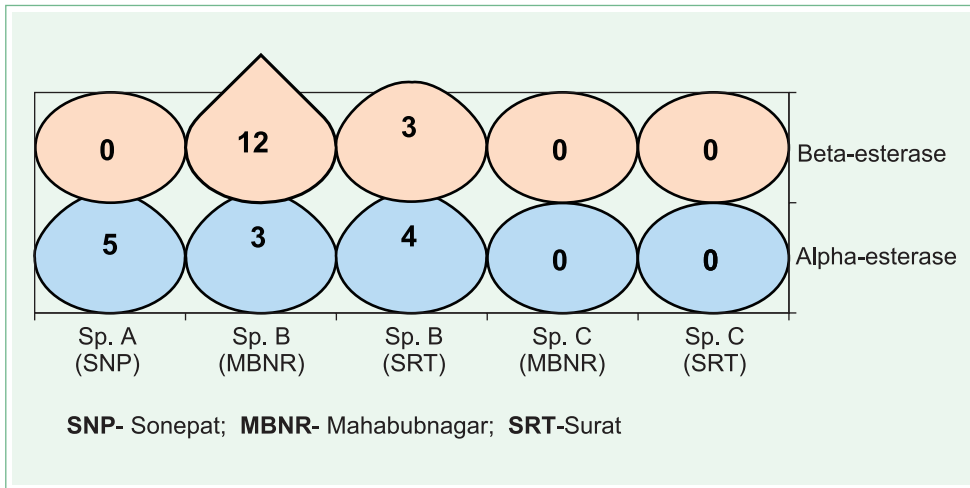


Fig. 44: Proportions of the sibling species populations showing activities below the observed threshold values (as observed for the susceptible strains) of esterases

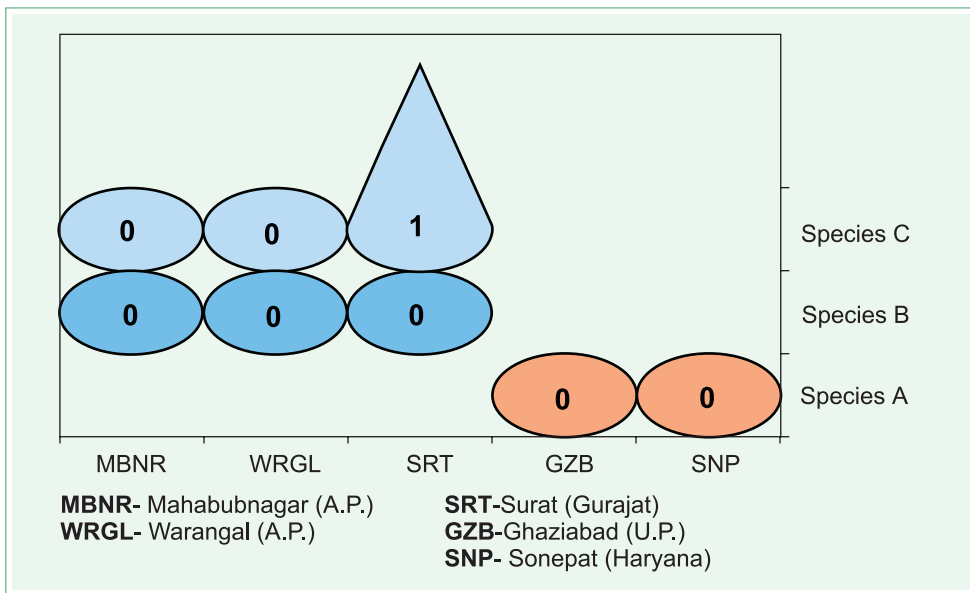


Fig. 45: Proportions of sympatric sibling species populations showing uninhibited activity above 100% activity of acetyl cholinesterases

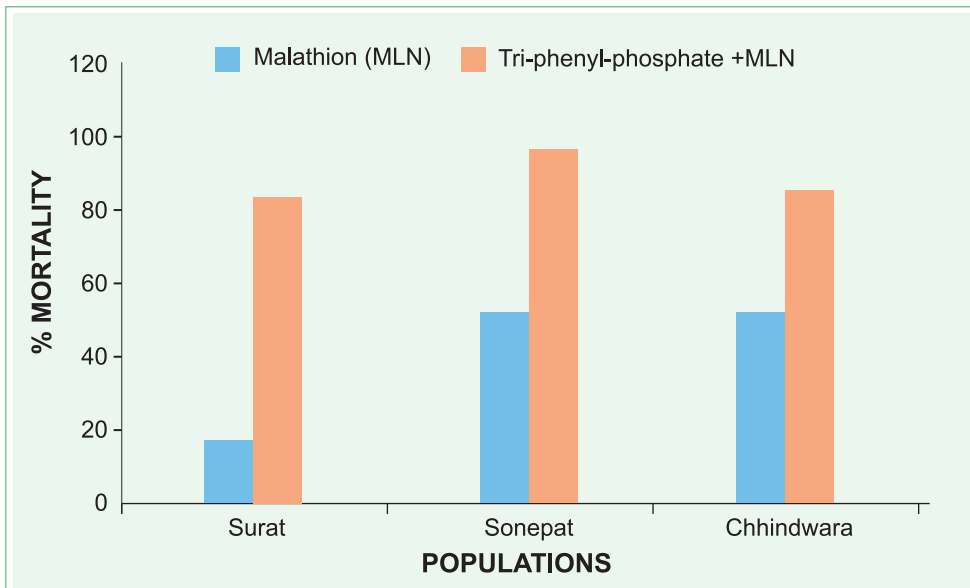


Fig. 46: Synergistic bioassays to determine the malathion resistance

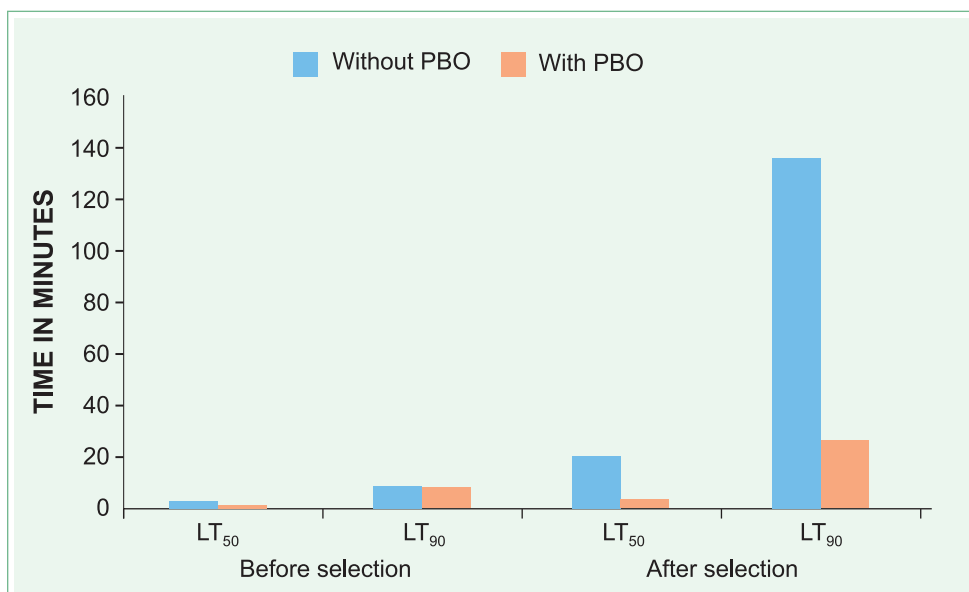


Fig. 47: Susceptibility of *An. culicifacies* Rameswaram (Rmr) strain to deltamethrin (0.05%) before and after laboratory selection with deltamethrin and in the presence of PBO

strain which confirmed the involvement of malathion carboxylestrase in malathion resistance in *An. culicifacies*. The selection of this mechanism results in narrow spectrum resistance and in such situation malathion can be replaced by 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. 47).

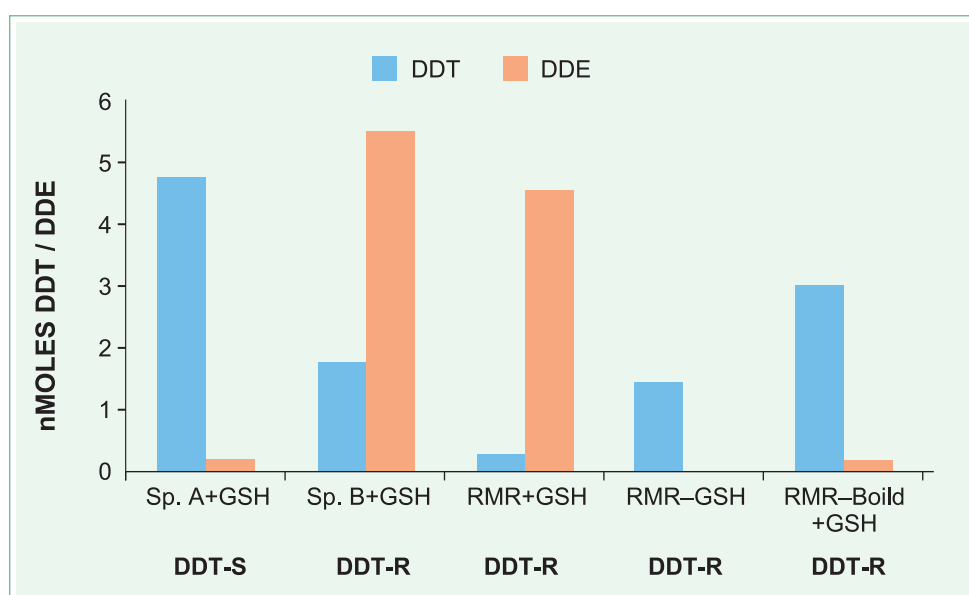


Fig. 48: Relative ratio of DDT metabolized to DDE *in vitro* in *An. culicifacies* with different susceptibility status to DDT and in the presence or absence of reduced glutathione (GSH)

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 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. 48).

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.

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