RESISTANCE IN *ANOPHELES CULICIFACIES* SIBLING SPECIES B AND C TO MALATHION IN ANDHRA PRADESH AND GUJARAT STATES, INDIA

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ABSTRACT. Studies conducted in Warangal, Khammam and Mahabubnagar districts in Andhra Pradesh and Surat district in Gujarat have revealed that *Anopheles culicifacies sensu lato* (*s.l.*) populations were resistant to malathion. In the absence of indoor spraying of malathion in public health programs in the 3 districts of Andhra Pradesh, resistance is attributed to the extensive use of pesticides in agriculture. Species B and C were sympatric in all areas surveyed, and both the species were resistant to malathion. In most of the surveys carried out in Mahabubnagar, Khammam and Warangal, levels of resistance were higher in species C than in B. In Mahabubnagar district an increase in resistance from 5.5 to 64% was observed from 1985 to 1987 in *An. culicifacies s.l.* The proportion of species C was low in the initial 2 surveys, and in the later surveys the proportion was almost equal to that of species B; the resistance level was also significantly higher than in species B. In Surat district, where resistance ranged from 74 to 93%, the level of resistance in the 2 species was almost the same.

INTRODUCTION

Anopheles culicifacies sensu lato (*s.l.*) is the major vector of malaria in the rural and periurban areas of India and neighboring countries. In India, insecticides such as DDT, HCH and malathion are being sprayed by the National Malaria Eradication Programme (NMEP) to interrupt the transmission of malaria. In spite of the continuous use of insecticides, malaria incidence remains high in several parts of the country. A major factor contributing to the resurgence of malaria was the development of resistance to insecticides in the vector species (Sharma 1984), especially in *An. culicifacies s.l.* (Bang 1985). In this species, resistance to DDT and HCH is reported for most parts of the country and to malathion in the states of Gujarat, Maharashtra and Madhya Pradesh. Malathion resistance in *An. culicifacies s.l.* in Andhra Pradesh state is considered primarily due to the use of agricultural pesticides (Subbarao 1979).

Anopheles culicifacies Giles has been cytotaxonomically identified as a complex of 4 sibling species: A, B (Green and Miles 1980), C (Subbarao et al. 1983) and D (Subbarao et al. 1988a, Suguna et al. 1989). In northern India, where HCH has been sprayed for the past 10 years or so, the 2 sympatric sibling species, A and B of the *An. culicifacies* complex, differ in their susceptibility to DDT both in the laboratory and field, with species A being more susceptible than species B (Subbarao et al. 1988b). These 2 sibling species differ in their seasonal prevalence (Subbarao et al. 1987), host feeding patterns (Joshi et al. 1988) and in their susceptibilities to *Plasmodium* infection (Subbarao et al. 1988c). A distinct pattern of distribution of the 4 sibling species occurs in India (Subbarao et al. 1988a). These findings encouraged us to study the responses of sibling species to other insecticides. The present study was carried out in Surat district in Gujarat and in Warangal, Khammam and Mahabubnagar districts in Andrha Pradesh. We examined the role of indoor spraying of malathion and usage of pesticides in agriculture in selecting for malathion resistance in *An. culicifacies s.l.*, and to detect differences in the responses of its sympatric sibling species B and C to malathion.

MATERIALS AND METHODS

In the state of Gujarat, Surat district, and in the state of Andhra Pradesh, Warangal, Khammam and Mahabubnagar districts, 2–3 villages per district were selected for mosquito collections. These were riverine villages with similar ecology. Female *An. culicifacies s.l.* resting on the walls, roofs and other objects in human dwellings and cattle sheds were collected with an aspirator and a flashlight. The mosquitoes were brought to the laboratory within an hour of collection in 30 × 30 × 30 cm cloth cages covered with wet towels. The resistance status of field-collected mosquitoes to malathion was determined by exposing *An. culicifacies s.l.* in batches of 20–25 mosquitoes to 5% malathion-impregnated papers for 1 h. Adults were held for a 24-h recovery period before recording the mortalities [World Health Organization (WHO) 1981]. Mosquitoes were also exposed to the WHO-recommended diagnostic dose for different intervals to plot dose-mortality response. To study the susceptibility of sibling species to malathion, semi-gravid females with ovaries in Christophers' stage III were used. Immediately after insecticide exposure, ovaries were removed

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Table 1. Response of *Anopheles culicifacies s.l.* and its sibling species from Andhra Pradesh and Gujarat states to 5% malathion impregnated papers.

STATE District Period of survey	All gonotrophic stages of *An. c. s.l.** [% survival/no. treated* (mean ± SD)]	Semi-gravid *An. culicifacies s.l.*^				
		Predominant sibling species composition**		Survivals (%) of		
		% B	% C	Sp.B ( )^^	Sp.C ( )^^	Chi-square values***
ANDHRA PRADESH						
Mahabubnagar						
Feb. 1985	5.5/100 (6.4 ± 2.2)	84.9 (79)	15.1 (14)	0 (0)	0 (0)	—
Apr. 1986	23/79 (23.0 ± 17.9)	98.3 (58)	1.7 (1)	23 (17)	0 (0)	—
Dec. 1986	58/165 (59.3 ± 10.5)	52.3 (56)	47.7 (51)	24 (53)	96 (48)	49.82 $P < 0.001$
Nov. 1987	64/585 (65.0 ± 14.2)	50.4 (232)	49.6 (228)	51.5 (167)	94 (141)	6.55 $P < 0.02$
Khammam						
Feb. 1985	53/230 (52.7 ± 11.3)	36.3 (29)	63.7 (51)	32 (25)	65 (46)	5.91 $P < 0.02$
Warangal						
Dec. 1985	90/225 (83.4 ± 8.2)	29 (20)	71 (49)	60 (20)	92 (49)	7.90 $P < 0.01$
Apr. 1986	43/122 (17.8 ± 13.1)	57.8 (37)	42.2 (27)	100 (9)	87.5 (8)	0.003 n.s.
Dec. 1986	51/127 (48.8 ± 20.5)	37.7 (20)	62.3 (33)	52 (19)	94 (32)	9.61 $P < 0.01$
Nov. 1987	97/134 (96.0 ± 2.8)	32.7 (18)	67.3 (37)	66.6 (3)	88.9 (9)	0.41 n.s.
GUJARAT						
Surat						
Apr. 1984	87.4/63^^^ (87.7 ± 5.3)	6.3 (4)	93.7 (59)	75 (4)	88 (59)	.00015 n.s.
March 1986	93/100 (93 ± 3.3)	11.5 (3)	88.5 (23)	100 (1)	100 (18)	—
Apr. 1987	74/226 (76.5 ± 11.9)	42.2 (43)	57.8 (59)	73 (15)	93 (15)	0.96 n.s.
Nov. 1987	86/469 (87.4 ± 15.2)	33.9 (292)	66.1 (569)	94 (50)	97 (77)	0.25 n.s.

* 20–25 *An. culicifacies s.l.* were treated per replicate. After 1-h treatment, ovaries from semi-gravid females were removed to establish the species composition in dead and alive mosquitoes.

** No. in parentheses are actual number of mosquitoes identified for sibling species. Data include both insecticide-treated and untreated populations.

*** Chi-square values tested for responses of sibling species.

^ Semi-gravid females in a collection generally vary from about 0 to 70% depending on the season.

^^ Total number of mosquitoes identified for the species both dead and alive mosquitoes.

^^^ Only semi-gravid mosquitoes were selectively exposed.

from both the live and the knocked-down mosquitoes and stored in vials with modified Carnoy's fixative (1:3, glacial acetic acid:methanol) for chromosome preparation to be used for the identification of sibling species. The standard procedure of holding the mosquitoes for 24 h post-exposure to the insecticide was not followed, as the mosquitoes would have gone beyond the suitable stage for species identification. Polytene chromosomes were prepared from the fixed ovaries using the method of Green and Hunt (1980). The diagnostic inversion genotypes, recognizable on the polytene chromosomes for the identification of sibling species, were: species B-Xab $2g^{1+h^1}$ and species C-Xab $2^{g^1} + h^1$ (Subbarao et al. 1983).

RESULTS

Malathion resistance in *An. culicifacies s.l.* was observed in all the collections made in Gujarat and Andhra Pradesh states, though in

varying levels (Table 1). In Andhra Pradesh, percent survival ranged between 5.5 and 64% in Mahabubnagar district and between 43 and 97% in Warangal district when exposed to 5% malathion-impregnated papers for 1 h in bioassay tests. In Khammam district, the percent survival was 53% in February 1985; in the later surveys, sufficient numbers of mosquitoes were not available to carry out the bioassay tests. Survival ranged between 74 and 93% in Surat district (Gujarat). In the above bioassay tests, mosquitoes were scored for mortality immediately after the exposure period as these insects were to be used for cytological identification of sibling species. However in November 1987 in a few replicates, mosquitoes were scored immediately after the exposure period, whereas in others they were scored after a 24-h recovery period. The percent survival in replicates with recovery period was 48% (n = 119), 85% (n = 66) and 83.5% (n = 144) in Mahabubnagar, Warangal and Surat districts, respectively, whereas in replicates without the recovery period, the percent survival was 64, 97 and 86%, respectively. The difference in mortalities between the 2 procedures used is significant for Mahabubnagar populations (χ^2 = 10.7 $P < 0.005$) and Warangal population (χ^2 = 10.05 $P < 0.005$) whereas it was nonsignificant for Surat (χ^2 = 0.59 n.s.). The percent mortalities were higher in replicates that were scored after the recovery period. This could be because the tests were carried out in the field where temperature and humidity could not be controlled.

For Mahabubnagar populations, LT_{50} and LT_{90} values were 69 min and 656 min, respectively, whereas for Surat populations they were 109 min and 517 min, respectively. In Khammam and Warangal, tests for dose-mortality curve could not be carried out due to nonavailability of the required number of mosquitoes.

For all areas in Andhra Pradesh and Gujarat, species B and C were found to be sympatric (Table 1) with the occasional occurrence of other sibling species. The proportion of species C was almost always higher, irrespective of the period of survey, in all districts except Mahabubnagar (Andhra Pradesh). In Mahabubnagar, during the first 2 surveys, the proportion of species B was 85–98%, whereas in the later surveys the proportion of species C increased and was almost equal to that of species B. Malathion resistance was observed in both species, but the percent survival of species C was found to be significantly higher than that of species B in some districts of Andhra Pradesh (χ^2 values in Table 1). In Surat (Gujarat), no significant difference was observed between the 2 species (χ^2 values in Table 1).

DISCUSSION

In Surat district, indoor spraying of malathion was introduced into the Public Health Programme in 1969 to interrupt malaria transmission, and in 1973 malathion resistance was reported in *An. culicifacies s.l.* (Rajagopal 1977). The high percentage of resistance observed in the present study further confirms the above report. In this district, there has been little usage of pesticides as there is no cash crop cultivation, and cereals and millets are cultivated only in limited areas. Hence, it seems likely that the selection for resistance to malathion in *An. culicifacies s.l.* in these areas is mainly due to indoor residual spray of malathion in public health programs. The high level of resistance to malathion observed in this species was not altered even when malathion was replaced by DDT on an experimental basis in 1986 and 1987 (NMEP directorate, personal communication).

In Warangal, Khammam and Mahabubagar districts in Andhra Pradesh, which were under a regular spraying of 2 rounds of DDT at 1 g/m^2 since the late 1950s, resistance to malathion was observed in *An. culicifacies s.l.* populations; i.e., resistance to malathion was observed in the absence of indoor residual spraying. In 1987, 3 rounds of malathion were sprayed for the first time at the rate of 2 g/m^2 in Khammam and Warangal districts. Thus, this study confirms the suggestion of Subbarao (1979) that malathion resistance in *An. culicifacies* might have resulted from the extensive use of pesticides in agriculture in Andhra Pradesh.

The pesticides used for the protection of chili peppers and cotton, the 2 major cash crops in Andhra Pradesh, are mono-crotophos, dimethoate, methyl parathion, endosulfan, malathion, fenthion, cypermethrin, deltamethrin, etc. As agricultural use of malathion is minimal in these areas (Directorate of Agriculture, Andhra Pradesh; personal communication) the possible reason for malathion resistance could be usage of related compounds with carboxylester bonds. However, *An. culicifacies s.l.* populations are still susceptible to other organophosphates and carbamates, viz, fenthion, fenitrothrion and propoxur (unpublished data). This suggests that the malathion resistance mechanism in *An. culicifacies* populations in the above districts may be exclusively carboxylesterase mediated, as has been observed in *An. culicifacies* B in Sri Lanka (Herath et al. 1987, Hemingway et al. 1988), *An. stephensi* Liston in Pakistan (Hemingway 1983), and *An. arabiensis* Patton in the Sudan (Hemingway 1985).

The low levels of resistance observed in Mahabubnagar district in Andhra Pradesh in 1985 and its gradual increase in subsequent surveys,

in contrast to the high levels observed in the surveys in Khammam and Warangal, suggest that the development of resistance in Mahabubnagar populations is probably of recent origin. In Warangal and Khammam, cash crop cultivation has been done since the 1950s and large areas are under cultivation. In Mahabubnagar, this practice was introduced in the early 1980s in limited areas. Differences in acreages under cultivation, amount of insecticides used and duration of selection pressure are common factors affecting resistance, as has been well documented in *An. albimanus* Wied. in El Salvador (Georghiou et al. 1974).

In Warangal and Surat districts, the levels of resistance developed in both the species was high. In Mahabubnagar, where the development of resistance to malathion is a recent phenomenon, the proportion of species C in 2 initial surveys was low, and hence response of this species to the insecticide could not be ascertained. But in the 2 later surveys, the proportion of species C in the population was almost equal to B, and the percent resistance in species C was 96 and 94%, respectively. The high resistance levels observed in species C compared with that in species B in the later 2 surveys suggest that development of resistance in species C probably is at a faster rate than in species B.

Since all the study areas have a similar ecology, it may be assumed that in all the areas species B could have been the predominant species before the onset of resistance, and then due to a rapid buildup of resistance to malathion in species C, the proportion of species C increased and remained high because of continuous selection pressure. Thus, data from Mahabubnagar suggest that the resistance is being selected at a faster rate in species C than in species B. This probably was a factor in the shift in proportions observed for the 2 sibling species. Similar observations were made in the case of species A and B under DDT selection pressure in Uttar Pradesh wherein the proportion of species B increased (Subbarao et al. 1988b).

This study provides additional evidence that pesticides used in agriculture can select for resistance in medically important vectors sharing the habitat of agricultural pests. In Sri Lanka, *An. nigerrimus* Giles and *An. subpictus* Grassi, whose larval habitats are rice fields, developed broad spectrum resistance (Hemingway et al. 1988); and in El Salvador, where cotton cultivation is intensive, *An. albimanus* developed resistance to malathion (Breeland et al. 1970) and other related insecticides (Georghiou et al. 1972). To prevent rapid buildup of resistance, in Sri Lanka malathion is used only for the control of malaria vectors and not for agricultural pests (Herath et al. 1987). At present there is no such policy in India. This study illustrates the importance of careful selection of pesticides for both agriculture and public health.

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