STUDIES ON THE NATURE OF MALATHION RESISTANCE IN A POPULATION OF *ANOPHELES STEPHENSI* FROM SOUTHERN IRAN

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ABSTRACT. Malathion resistance in a population of *Anopheles stephensi* originating from Bandar Abbas, southern Iran was found to be intermediately dominant in its expression. Repeated backcrossing accompanied by selection with a dosage of malathion known to kill most susceptibles yielded results not entirely consistent with the resistance being dependent on a single gene mechanism, though the use of synergists indicated the involvement of carboxylesterase only.

INTRODUCTION

Manouchehri et al. (1975) reported on the laboratory selection of malathion resistance in *Anopheles stephensi* Liston from 2 localities in Iran from progeny of the survivors of the exposure of adults to 3.2% malathion for one hour. The actual existence of malathion resistant individuals of this species in the field in Bandar Abbas, Iran, was reported by Manouchehri et al. (1976a) and subsequent increasing trends in this resistance by Manouchehri et al. (1976b).

This paper reports on a laboratory study of the nature of this malathion resistance in a population of *An. stephensi* originating from Bandar Abbas, southern Iran.

MATERIALS AND METHODS

The strains of *Anopheles stephensi* used were:

ST/15—a standard strain derived from a population originating from Delhi, India in about 1947 and presumed to be susceptible to all insecticides.

SM35 and E316—sub-colonies derived from a population ST/ROK originating from Roknabad, Minab, Bandar Abbas, southern Iran and supplied by Dr. A. V. Manouchehri of the Department of Envi-

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ronmental Health, School of Public Health, University of Teheran in 1976.

Malathion impregnated papers (5% in olive oil/ionol solution) used for testing the adults were supplied by the World Health Organization. The synergists triphenyl phosphate (TPP) and piperonyl butoxide (PB) were provided by Dr. F. J. Oppenoorth of the Laboratory for Research on Insecticides, Wageningen, Holland. *S,S,S*-tributyl phosphorotrithioate (DEF) was supplied by Dr. R. M. Sawicki of Rothamstead Experimental Station, Harpenden, England. The impregnated papers of these were prepared locally at the maximum non-toxic dosage of the appropriate synergist.

Mosquito rearing, maintenance and the experimental procedure were carried out under controlled conditions of temperature (25–28°C) and RH (70–80%). Standard rearing procedures adopted for anopheline species were followed. Testing procedures involved the standard WHO adult susceptibility test using less than 1 day old adult males and females.

In the ST/ROK population the proportion of malathion resistant individuals was low at the time of receipt from the field. There was 99% mortality after 1 hour's exposure to 5% malathion among a sample of 246. Selection was therefore continued in the laboratory, but on a very gradual basis with a view to maintaining the genetic variability of the population, knowing it to have been derived in the first place from a very few eggs. The progeny from the eggs from the field were maintained over a number of generations until a desired population size was reached. In each of the following 5 generations, a sample of the population to be selected was exposed to 5% malathion for 15 min and survivors were returned for mating with the unexposed stock population. In each of the next 5 generations the entire population was treated with the same dosage of malathion. The process of initially exposing only a proportion, followed by exposure of the entire population, was repeated gradually increasing the exposure time. Exposures of 30 min, 1 hr and 2 hr to 5% concentration were involved with a minimum of 5 generations between each extension of exposure time. Initially when selecting, particularly at 1 and 2 hr exposures, the survivors were small in number. This necessitated generations of unselected maintenance until the densities were re-established to enable continued selection. It was expected that such a selection procedure would avoid any rapid elimination of background genetic material which might eventually contribute to the stabilization of resistance in the population. In addition, such partial selection may simulate to some extent the type of selection that might occur under field conditions. The resulting population was maintained unselected for 3 more generations and designated SM35. It showed 11% mortality after 1 hr exposure to 5% malathion among a sample of 616.

The E136 sub-colony was derived from SM35 by single family selection. The process of sib-mating (brother-sister mating) accompanied by susceptibility testing was followed with a large number of families until one was encountered where all the adults emerging from an egg batch of a single female gave no mortality after one hour's exposure to 5% malathion. Further sib-matings from this family were followed through 3 successive generations before 8 families showing no mortality after exposure to 5% malathion for 1 hr were pooled to give strain E136 which was then continually pressurized with malathion to ensure full homozygosity.

To study the mode of inheritance of the resistance, the ST/15 and E136 strains and the progeny of the cross between them were exposed to 5% malathion for 1 hour. The hybrid offspring were also backcrossed to ST/15, the progeny being exposed to the same dosage of malathion. The survivors were again backcrossed to ST/15 and their offspring again exposed to 5% malathion for 1 hour. This procedure was repeated for 3 successive

backcrosses in an attempt to distinguish monofactorial from polyfactorial inheritance.

To gain some indication of the detoxication mechanisms involved in the resistance, a sample of the SM35 population was initially exposed to an appropriate synergist and subsequently to malathion. At the same time, a comparable sample of the same population was tested with malathion by itself.

RESULTS AND DISCUSSION

A sample of 1,054 adults of the ST/15 population exposed to 5% malathion for 1 hr produced a 98% mortality. The exposure of 80 adults of the E136 strain to the same dosage produced no mortality at all. The F_1 progeny of the cross between them on the other hand showed 28% (among a sample of 111) and 31% mortality (among a sample of 120) from the reciprocal matings. On this basis malathion resistance in the E136 population can be considered to be intermediately dominant in its expression and thus differs from the almost completely dominant nature of malathion resistance reported for a population of *An. culicifacies* Giles from India (Herath and Davidson 1981a).

Five percent malathion for 1 hr discriminates susceptibility from homozygous resistance but not from the heterozygous state. Taking into consideration the average mortality of 29.5% of the heterozygotes and the 2% survival of the susceptibles at this dosage, the mortalities in the backcross progenies would be expected to remain at about 64% in consecutive backcrosses with selection if inheritance is dependent on a single genetic factor.

Tables 1 and 2 analyze the data from 7 families of the first backcross and 8 of the second. In the first backcross (Table 1) all 7 families show a significant departure from the expected mortality of 64%, 6 of them showing less than this figure. The overall mortality was in fact only 42%. In the second backcross (Table 2) 3 out of 8 families produced the results expected of a single gene hypothesis but 5 of them did not. Half the families showed less than the expected mortalities though the overall figure was the expected one of 64%. A third backcross was made but the offspring were reared together instead of in single families. Here of 858 adults tested 382 died, a mortality of 45%, again a significant departure from the expected ($\chi^2 = 101.60$; $P = <0.01$).

Table 1. Single family results of exposure to the discriminating dosage of malathion of the offspring of the first backcross of hybrid (resistant Iranian x susceptible Indian) to the susceptible Indian population of *Anopheles stephensi*.

Family number	Number tested	Number dying	χ^2 (1:1 expectation)
1	123	47	25.92*
2	63	29	6.06*
3	49	40	5.22*
4	73	27	17.02*
5	55	21	11.20*
6	107	37	28.26*
7	40	14	11.08*
Total	510	215	75.58*

* $P = < 0.05$.

Table 2. Single family results of exposure to the discriminating dosage of malathion of the second backcross progeny involving resistant and susceptible *Anopheles stephensi* populations from Iran and India.

Family number	Number tested	Number dying	χ^2 (1:1 expectation)
1	320	204	0.01
2	336	205	0.96
3	186	133	3.30
4	90	36	16.68*
5	110	56	5.60*
6	105	86	10.78*
7	187	165	33.74*
8	266	134	15.24*
Total	1600	1019	0.048

* $P = < 0.05$.

These results are not consistent with the single gene hypothesis, nor do they strongly indicate polyfactorial inheritance, as there is no consistent rise in mortality over the 3 successive backcrosses. However, results from the synergist work favor a single gene interpretation (see Table 3). If a single gene is involved in this resistance the departures from the expected values may have been caused by a loss of susceptibles during the rearing procedure. However, individual family yields of pupae from eggs were not recorded (as they were in the case of similar studies with *An. culicifacies* (Herath and Davidson 1981a)).

Pre-treatment of the SM35 population with TPP produced strong synergism with malathion suggesting carboxylesterase (CE) involvement in malathion resistance (see Table 3). DEF produced no synergism. With PB there was continuous antagonism at all the dosages tested. This could be attributed to the inhibition of mixed function oxidases (mfo's) involved in the oxidative conversion of the P=S bond to P=O during the activation of malathion to the toxic malaoxon. There was no evidence to suggest any mfo involvement in malathion detoxication such as was found in multiple resistant populations of *An. culicifacies* (Herath and Davidson 1981a) and *An. albimanus* Wiedemann (Herath and Davidson 1981b).

Table 3. Results (percentage mortalities) of exposure of the malathion resistant SM35 population of *An. stephensi* to malathion alone and to malathion following pretreatment with the synergists TPP, DEF and PB.
(Figures in parentheses denote the number of mosquitoes tested).

Insecticides &/ synergist	Exposure time in minutes						
	15	30	45	60	90	120	240
Malathion 5%	0(120)	3(95)	10(198)	18(562)	50(70)	75(391)	96(104)
TPP + a-malathion	17(111)	69(86)	97(105)	96 (72)	—	99(104)	—
DEF + malathion	—	0(52)	—	2 (95)	23(65)	—	—
PB + malathion	—	—	—	4 (96)	—	0(106)	93 (98)
Control + malathion	2 (50)	—	19(118)	10 (87)	—	68(142)	—

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