BEHAVIOUR CHANGES IN PERMETHRIN-RESISTANT STRAIN OF *ANOPHELES* STEPHENSI

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Abstract - Behaviour studies indicated that the permethrin-resistant strain of An. stephensi was 3-fold resistant to knock-down compared with the susceptible strain. The resistant strain was however 3-fold less irritable to permethrin and less responsive than the susceptible strain to the movement of an aspirator. If reduced irritability and reduced responsiveness to catch are consequences of the changes in the nervous system, then such a form of resistance may be disadvantageous to mosquitoes in natural populations.

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INTRODUCTION

Anopheles stephensi is known to be an important malaria vector in the Persian Gulf, the Middle-East and Indian subcontinent. As a result of the continuous application of insecticides in these regions, *An.stephensi* populations are known to be resistant to DDT (Davidson, 1958; Davidson & Jackson, 1961; Mofidi et al., 1958), dieldrin (Davidson & Mason, 1963; Zaim, 1987) and malathion (Manouchehri et al., 1974, 1975, 1980); Rathor & Toqir, 1980; Hemingway, 1983; Scott & Georghiou, 1986; Herath & Davidson, 1981; Hemingway, 1980; Hemingway et al., 1984. There are some reports of pyrethroid resistance in *An. stephensi* based on laboratory selection (Omer et al., 1980; Malcolm, 1988a; Chakravorthy & Kalyanasundaram, 1992; Sahgal et al., 1994)

MATERIALS AND METHODS

Permethrin; 3-phenoxybenzyl-(1R)-cis, trans-3(2, 2-dichlorovinyl)-2, 2, dimethyl cyclopropanecarboxylate, technical grade 96.2%, density = 1.2 and cis/trans ratio 40/60.

Mosquito strains used

The main *An. stephensi* strains used throughout this work are as follows:

DUB-APR; a sub-strain from DUB-S, selected with permethrin at the adult stage in the insectary. IND-S; a strain susceptible to permethrin, and a standard laboratory colony originating from New Delhi in 1947.

Insecticide testing method

Tests on adults were carried out according to the methods of WHO (1970). At each exposure time 50-400 mosquitoes representing 2-16 individual replicates of 25 adults were tested. To reduce variability in the replicates, 2-3 day old sugar fed adults were used. Due to the knockdown effect of pyrethroids on the adults, the exposure tubes were held in a horizontal position during tests. The mortality rate was scored after a 24 h recovery period. Insecticide exposure took place in a room with a temperature of 25±1C and holding tubes were held in a insectary under controlled conditions of 27 ± 1 °C and 75±5% relative humidity. Permethrin was studied in the laboratory for its comparative efficacy against females of resistant and susceptible strains of *An.stephensi*. Initially the susceptibility of DUB-APR, and IND-S strains was tested with 10 $\mu g/cm^2$ impregnated papers. To eliminate the importance of early knock-down of adults during exposure, testing tubes were held in a horizontal position (WHO, 1992). In each test 2-3 day-old females were tested with fresh laboratory-made impregnated papers. Regression line parameters including intercept (a), slope ± standard error (b ± SE), heterogeneity about the regression line with degrees of freedom [X^2(df)], LT5O ± 95 % confidence interval (LT50 ± 95% C.I.), LT90 ±95% confidence interval (LT90 ± 95% C.I.) were determined from the dosage mortality regression lines as described by Finney (1971). LT50 and LT90 represent respectively the times of exposure required to kill 50% and 90% of the population at a given concentration of pesticide. The LT50 is the main parameter used in this study to assess the relative toxicity of pesticides.

Statistical methods

Dosage mortality regression lines were determined by the probit analysis method of Finney (1971), using the Probit 79 programme on an IBM computer. Goodness of fit of the points to a straight line were tested by Chi-square (χ^2) analysis. Other statistical analysis were determined by Minitab and Glim programme on an IBM computer.

Impregnated nets

Impregnation of netting materials was carried out as described by WHO (1985). Pieces of netting were cut and the area of the net to be treated calculated. The amount of water necessary to saturate but not run off the net was determined by weighing dry pieces and then dipping in water. The excess water was squeezed out gently before weighing again. The difference in the two weights gave the amount of water absorbed by the net. The amount of permethrin needed to treat a net was measured. The pieces of netting were dipped into prepared emulsion of permethrin in a non-absorbent container, the treatment being carried out with care to cover all surfaces, before the net was partially unfolded on a non-absorbent surface to air dry. Netting pieces with dosages of 500 mg/m^2 of permethrin were prepared.

Knock-down behaviour testing method

The knock-down behaviour of females of DUB-APR and IND-S strains exposed to 500 mg/m^2 permetbrin impregnated net was determined by using WHO plastic bioassay cones. Each cone was attached to a piece of netting using an elastic band, the hole in the cone was stopped with cotton wool. Five female mosquitoes were released in to each cone. Mosquitoes were left in the cone and the time taken for each mosquito to be knocked-down was recorded. Mean and standard deviation of the first knock-down, the last knock-down and the median knock-down were recorded. For each strain 100 mosquitoes were tested represented in 20 replicates. Knockdown results for each individual female of both resistant and susceptible strains were graphically plotted.

Irritability testing method

The level of irritability of mosquitoes was measured according to the method described by WHO (1963). 20 unfed 2-3 day old females of each strain (resistant and susceptible) were individually exposed to 10 μg/cm^2 =0.25% permethrin in an exposure chamber and the number of take-offs were counted during a 15 minute exposure time. The differences between number of take-offs for (RR) and (SS) strains were calculated. The mean and standard deviation of number of take offs for individuals of each strain were calculated.

Results

The results of the studies on the efficacy of permethrin against females of *An. stephensi* have been summarized in Table 1. The LT50 value from Table 1 showed that DUB-APR (strain selected with permethrin at the adult stage) is 8-fold more resistant to permethrin than the IND-S strain which remains susceptible.

Knock-down behaviour study

We have established that in a permethrin resistant strain of An. step hensi PB and TPP reduced the level of resistance to permethrin (vatandoost et al., in preparation). Since resistance was not completely eliminated by these synergists, it was speculated that other mechanisms were involved in resistance in addition to metabolism. It has been known for some time that nerve membrane is the primary target of pyrethroid insecticide (Narahashi, 1976). A number of later studies have suggested that the voltage-gated nerve membrane sodium channels may be involved in the kdr mechanism (Dong & Scott, 1991; Bloomquist, 1996).

In this study knock down of females of permethrin-resistant (DUB-APR) and susceptible (IND-S) strains of An. step hensi was quantitated. The experimental methods are described by former workers. In summary, into each bioassay cone attached to netting impregnated with 500 mg/m^2 permethrin, five females were released and the knock-down time of each individual mosquito was recorded. The experiment was carried out with 100 mosquitoes of each strain, representing 20 replicate tests of 5 mosquitoes. The knock-down time of each mosquito was recorded. The results are presented in Table 2 and Fig. 1. Adult females of IND-S strain showed first knock-

Table 1. Probit regression line parameters for adult females of *An.stephensi* tested with permethrin at 10 μg/cm^2

Strains	a	b ± SE	LT50 ± 95% C.I.	LT90 ± 95% C.I.	χ^2 (df)	P
			77.4	126.5		
DUB-APR	-10.70	5.57 ± 0.41	82.0	139.2	4.95 (5)	
			87.0	157.4		> 0.05
			9.4	19.4		
IND-S	-3.84	3.74 ± 0.42	10.6	23.4	5.21 (3)	
						> 0.05
			12.1	30.4		

Table 2. The mean time to knock-down in minutes of females of DUB-APR and IND-S strains of *An.stephensi* exposed to mosquito netting impregnated with 500 mg/m^2 permethrin time in minutes to knock-down of mosquitoes 1-5

Strain	1	2	3	4	5	Mean ± SE
IND-S	5.1 ± 0.18*	5.6 ± 0.22	6.0 ± 0.18	6.6 ± 0.18	7.3 ± 0.24	6.1 ± 0.20
DUB-APR	14.5 ± 0.50	17.0 ± 0.64	19.6 ± 1.07	23.8 ± 1.33	30.5 ± 1.77	21.0 ± 1.0

Each test comprised 5 mosquitoes in 20 replicates

* ± SE

Table 3. Irritability level of females of resistant and susceptible strais of *An.stephensi* to permethrin

Strains	No. of take-offs/20 female/15 min	No. of take-offs/female /15 min	No. of take offs/female /min ± SD
DUB-APR	254	12.7	0.846 ± 0.52
IND-S	706	35.3	2.353 ± 1.35

Table 4. Analysis of variance to compare irritability level of resistant and susceptible strains of *An.stephensi* to permethrin

Source	df	SS	MS	F	P
Factor	1	17.1	17.1	16.37	P< 0.0001
Error	28	29.25	1.04		
Total	29	46.35			

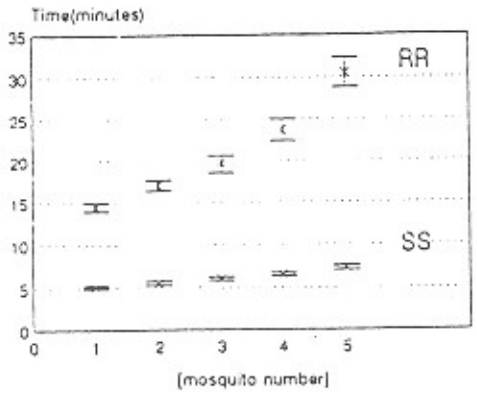

Fig. 1. Knock-down behaviour of females of resistant and susceptible strains of *An.stephensi* to permethrin impregnated net. Vertival bats = SE

Irritability study

Experiments were performed on the IND-S and DUB-APR strains to determine the irritability level of adult females exposed to permethrin impregnated paper. The method used was suggested by according to WHO (1963). In summary 20 unfed 2-3 day old females of each strains were individually exposed to 10 μg/cm^2 permethrin in an exposure chamber and the number of take-offs were counted during 15 minutes exposure time. The difference between susceptible and resistant strains in terms of irritability to permethrin was determined by analysis of variance.

The results of irritability tests are summarized in Table 3 and illustrated in Fig. 2. During the exposure time of 15 minutes, the mean number of take-offs per female were 12.7 and 35.3 for DUB-APR and IND-S strains respectively. An analysis of variance of the number of take-offs for the two strains showed that DUB-APR is significantly less irritable to permethrin that the IND-S strain, F= 16.37 (1.28) P <0.0001 (Table 4). The resistant strain was 2.8 times less irritable to permethrin than the susceptible strain as measured by the number of take-offs.

Responsiveness to external movement

Rowland (1991) reported some behavioural changes in cyclodiene resistant strains of *An. stephensi*, for instance egg production for oviposition, life time fecundity and flight activities of resistant females were less than those of susceptible strain.

Pyrethroid insecticides share many characteristics with DDT and DDT analogues, including knock-down and killing activity resulting from action against sodium channels of the peripheral and central nervous system (Zebra, 1988). In resistant strains various physiological mechanisms change the behavioural characteristics of the nervous system receptors and increase their permeability to chloride ions, causing hyperinhibition of the nervous system (Rowland, 1991).

In order to carry out further investigations on the behavioural changes in the permethrin resistant strain, the responsiveness of IND-S and DUB-APR genotypes to external movement were determined. The experiments are described in detail earlier . In summary, volunteers were allowed to catch the populations of known number of resistant and susceptible mosquitoes from two unmarked cages. The experiments were repeated 15 times for each strain and the times of aspirator catches recorded. The results are

shown in Table 5. Average time required to catch IND-S strain was 183.4 seconds, compared with DUB-APR which needed 118.2 seconds for collection. Two-way analysis of variance in Table 6 indicated that IND-S strain is more responsive than DUB-APR strain and was able to detect movement of the aspirator and took longer to catch.

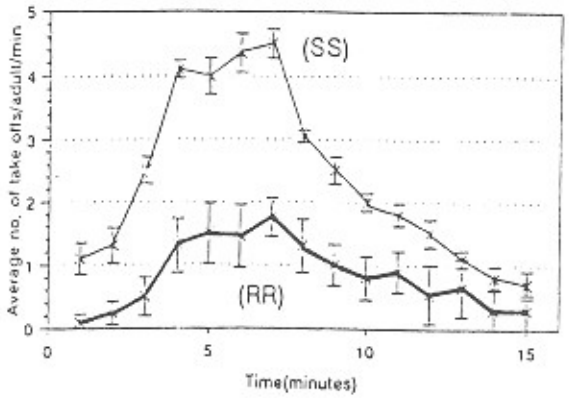

Fig. 2. Irritability level of females of resistant and susceptible stratins of *An.stephensi* to permethrin impregnated paper

Table 5. Responsiveness of resistant and susceptible genotype of An.stephensi to external movement. Experiment was carried out with equal numbers of 10 females in each cage and time of aspirator catch was recorded

Replicates	IND-S	DUB-APR
1,2,3	194	101
4,5,6	193	105
7,8,9	169	129
10,11,12	178	126
13,14,15	183	130
Mean	183.4	118.2
SD	10.5	14.0

Table 6. Analysis of variance of responsiveness of resistant and susceptible strains of *An.stephensi* to external movement

Source	DF	SS	MS	F	P
Responsiveness	1	10628	10628	151	P< 0.0001
Block	4	103	26	0.09	N.S
Error	4	1125	281		
Total	9	11856			

DISCUSSION

The knock-down behaviour of resistant and susceptible strains which is summarized in Table 2 and illustrated in Fig. 1, revealed that the resistant strain is 3.4-fold resistant to knockdown compared to the susceptible strain. As in our study, knock-down behaviour of pyrethroid-resistant and susceptible strains of larvae of *H. irritans* was determined when larvae were exposed to filter paper treated with permethrin (Crosby et al., 1991). Their study showed that resistant horn fly larvae were 42-times more resistant to knock-down than the susceptible larvae. Neurophysiological studies demonstrated that the basis for kdr is a reduced sensitivity of the nervous system to the neurotoxic action of pyrethroids. Magesa et al. (1994) exposed the DUB-APR and BEECH strains of *An. stephensi* for 2 minutes to a range of doses of permethrin on nylon netting and knock-down was measured 1 h after treatment. A resistance ratio of 370.1 was observed and exposure for 2 minutes to 200 mg/m^2 almost discriminated between the two strains.

Irritability studies of resistant and susceptible strains, the results of which are shown in Tables 3 and 4 and Fig. 2, indicated that the resistant strain is 2.8 times less irritable to permethrin in comparison with susceptible ones. In contrast to our irritability results, Lockwood et al. (1985) have found that pyrethroid-resistant populations of the horn fly were significantly more irritated by permethrin at lower doses than the susceptible populations. However, the form of behavioural resistance to deltamethrin was different from that found with permethrin. The threshold for response in the resistant strain was increased with deltamethrin. They concluded that the behavioural response of resistant horn fly to permethrin would be selectively advantageous. In populations of horn fly two classes of behavioural resistance are recognized: stimulus-dependent, which requires sensory stimulation of the insect for avoidance to occur. Stimulus-dependent behavioural resistance includes irritability, in which an insect is stimulated to leave a toxic environment upon contact, or before contact with a treated surface (Lockwood et al., 1984). Stimulus-independent behavioural resistance generally involves some forms of exophily, in which an insect avoids exposure to the toxicant by prolonged occupation of a nontoxic habitat (Lockwood et al., 1984). In populations with stimulus-independent behaviour the resistant strain uses some cue (other than the toxicant) to identify consistently untreated habitats (Byford et al., 1 987b).

In order to carry out further investigation on behavioural changes in terms of responsiveness to being caught with an aspirator the method of Rowland (1991) was followed. Results from Table 4 showed that the resistant strain is less responsive (1.5-fold) than the IND-S strain to the movement of an aspirator and this is in agreement with the study of Rowland (1991) who found that cyclodiene-resistant *An. stephensi* are 1.6-fold less responsive to movement. Many resistance studies have demonstrated a negative association

between behavioural (measured as irritability) and physiological resistance (Sparks et al.,1989). Trapido (1954) and Georghiou (1972) have postulated that insects must be physiologically susceptible to a pesticide to evolve stimulus-dependent behavioural resistance and that, as physiological resistance evolves, behavioral resistance declines. In our study the physiologically resistant strain of *An. stephensi* (DUB-APR) was shown to be at a behavioural disadvantage and fitness costs may be associated with physiological resistance.

Studies of the response of resistant and susceptible strains to permethrin-impregnated netting (500 mg/cm^2) showed that the resistant strain is 3.4-fold resistant to knock-down compared to the susceptible strain. The resistant strain was found to be 2.8-fold less irritable to permethrin. These results parallel those of Lockwood et al. (1985) who found that pyrethroid-resistant populations of horn fly are significantly less irritated by deltamethrin at low doses than are susceptible populations. However, the form of behavioural resistance to permethrin was the opposite to that of our irritability study (i.e. pyrethroid-resistant horn flies were irritated more rapidly by permethrin than the susceptible flies). They concluded that a behavioural response of resistant horn fly to permethrin would be selectively advantageous to the fly. Under field conditions, for a susceptible population to survive, rapid irritation is necessary so as to prevent the acquisition of a lethal dose upon contact with treated surfaces. In contrast, in DUB-APR strain physiological resistance to permethrin resulted in a decline in irritability to this insecticide. Thus resistant mosquitoes will spend more time in the treated surface than the susceptible strains, resulting in the acquisition of more insecticide. Sparks et al. (1989) have demonstrated a negative association between behavioural (measured as irritability) and physiological resistance. Trapido (1954) and Georghiou (1972) have postulated that insects must be physiologically susceptible to a pesticide to evolve stimulus-dependent behavioural resistance and that, as physiological resistance evolves, behavioural resistance declines.

To further investigate behavioural changes the method of Rowland (1991) was followed. Results showed that the resistant DUB-APR strain is less responsive (1.5-fold) than the INDS strain to the movement of an aspirator and this is in agreement with the study of Rowland (1991) who found that cyclodiene-resistant *An.stephensi* is 1.6-fold less responsive to movement. Moreover he pointed out that resistant females were less responsive to oviposition stimuli, they produce fewer eggs per unit of blood, they fly less during the periods available for seeking hosts or oviposition sites, they seem incapable of responding to or taking advantage of 'moonlight' by prolonging activity, they respond more slowly to 'predators' and resistant males were less successful at competing for females than were those of a susceptible strain.

These studies of irritability and responsiveness to movement showed that the resistant strain of *An.stephensi* (DUB-APR) was less active than the IND-S strain. In the confines of a laboratory population cage this changed activity would be of little importance, but in nature resistant females would be at a great fitness disadvantage if they spent less time searching for hosts or good oviposition sites or if they were less responsive to predators. Behavioural disadvantage and fitness costs may be associated with such resistance. The link between these behavioural characteristics and resistance is presumably closely connected with the neurophysiological basis of the resistance mechanism that is, the reduced irritability and reduced activity in the DUB-APR strain seems to be a consequence of the reduced sensitivity of the nervous system recorded in the neurophysiological studies.

The mode of behavioural resistance that occurs in the field is extremely important as regards to management strategies. If behavioural resistance is due to a physiologically potentiated mechanism (stimulus-dependent), management of behavioural resistance is simply a matter of increasing physiological susceptibility. However, if behavioural resistance occurs through an independent selected mechanism (stimulus-independent), then increasing toxicity may do nothing to increase a compound's efficacy in the field. Stimulus-independent behavioural resistance as reported in horn fly populations by Byford et al. (1987c), is effectively resistance to an application system, i.e., the insect avoids a consistently toxic habitat without interacting with any particular toxicant. Therefore, in such a situation, if the choice of the resting places continues to evolve, the application of insecticide will become ineffective, regardless of the insecticide being used.

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