PERMETHRIN SELECTION ON THE ADULTS OF *ANOPHELES STEPHENSI* FROM BANDAR-ABBAS, SOUTH OR IRAN

H.Ladonni[1], PhD

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Abstract

A wild strain of *Anopheles stephensi* Liston (BAN-PR), originating from Bandar-Abbas, south of Iran, was submitted to selection with permethin at the adult stage. Ten successive generations of permethrin selection on the adults resulted in only 2.2 fold increase in tolerance. Crossing experiments on the selected and unselected strains indicated that the gene/genes for observed tolerance is inherited as an intermediate character with no indication of sex linkage. Synergist test on the selected strain indicated that oxidative detoxification might be the major mechanism in the selected strain for the observed tolerance. The relationship between resistance to DDT, dieldrin and malathion in permethrin selected strain was studied. The results indicated that there might be a positive correlation between resistance to dieldrin, malathion and permethrin in *An.stephenesi* from Bandar - Abbas.

1- Medical Entomology Department, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.P.O. Box 14155-6446

Introduction

DDT and pyrethroids are two categories of insecticide that share a number of similar properties. They are the only insecticides that possess a clear negative temperature coefficient of action on nervous system; they have two types of effect on insects; an initial rapid knock-down (kd) effect and a subsequent lethal effect. Both DDT and pyretroids act on the peripheral nervous system as well as on the central nervous system. The most compelling evidence for this similarity action comes from genetics. Busvine (1951) documented cross-resistance to pyrethrum in an Italian strain of housefly *Musca domestica*, that was resistant to knock down by DDT. The relationship between DDT and pyrethroids has been widley studied in housefly than any other pest species . Kdr factor is one of the important resistance factors, conferring resistant to pyrethroids, cross- resistance to DDT and greatly delay knock-down (3). The gene(s) conferring DDT and pyrethroid knock-down resistance (kdr) have been shown to be not a metablic factor, but is more likely to involve target site insensivity to pyrethroid -DDT action in insect nerves(2).

Kdr-type resistance, have been reported in a number of anophelinae and culicinae mosquitoes as the results of DDT and permethrin selection (8,6), but cross-resistance to DDT as the result of permethrin application and vice versa and kdr type resistance in the field are limited to only few mosquito species (12,6).

Among the culicinae, only two cases of pyrethroid resistance as the result of DDT resistance has been recorded. In the larvae of *Culex tarsalis* a kdr-type resistance (9) and in the adults of a strain of *Aedes aegypti* , a metabolic breakdown mechanism (10) were postulated for the observed resistance.

Among the anophelinae, surprisingly only two species, *An.albimanus* and *An.sacharovi* have been shown resistant to pyrethroids and low levels of pyrethroid tolerance in 6 species have been reported (12,6).

In south of Iran, *An.stephensi* has been known to be resistant to DDT, dieldrin and malathion(6). After appearance of malathion resistance in *An.stephensi* , propoxur has been substituted since 1977 as a residual insecticide in malaria control programmes. Recently pyrethroids have received considerable attention as candidate chemical for house spraying in malaria control programmes. The purpose of this study was to investigate the effect of permethrin selection on a wild strain of *An.stephensi,* from Bandar-Abbas, south of Iran; the relationship between resistance to DDT, permethrin, and dieldrin; and the inheritance of permethrin resistance.

Materials and Methods

Mosquito strains: the following strains of *An.stephensi* were used in this investigation:

BAN-S strain, a wild strain based on adults, collected from Chellow village, Bandar-Abbas , south of Iran in April 1991.

BAN-PR strain , a sub strain from BAN-S, selected with permethrin at the adult stage.

TEH-S strain , a laboratory stock of *An.Stephensi,* resistant to DDT and dieldrin, which has been maintained in an insectary at the School of Public Health, Tehran University of Medical Sciences for at leat 30 years.

WHO standard impregnated papers such as DDT and dieldrin were supplied by WHO , but permethrin, malathion and synergists impregnated papers were prepared in our laboratory by spreading 1.4 ml of a known volume of insecticide (technical grade) or synergist in acetone and relevant solvent by means of a pipet onto Whatman No.1 filter paper (12x15 cm). Olive oil was used as solvent for malathion and DMC (chlorofenethol), and silicon oil for permethrin and PB (piperonyl butoxide) impregnated papers.

Mosquito rearing and maintenance was carried out in the insectary at 26-28 C and 70-80% relative humidity, with a 12 hour light and 12 hour dark period. Tests on the adults were carried out in the insectary according to the method recommended by WHO(13), using 2-3 days old sugar fed female and males. Due to the knock-down effect of permethrin on the adults, exposure and holding tubes were placed in a horizontal rather than the normal vertical position. The adults were exposed to permethrin and knockdown count was made during the exposure time.

In order to study the mechanisms of DDT and permethrin resistance, the adults were pretreated with a sublethal dose of synergists, i.e, PB and DMC, for 45 minutes before treatment with insecticide.

Permethrin selection was carried out on adult males and females of the BAN-PR, a sub-strain derived from the BAN-S strain. The sexes had been separated at the pupal stage. The males and females were exposed separately and the survivors were released into a cage and allowed to mate. Permethrin selection was carried out using exposure time to produce about 80-90% mortality.

To obtain the hybrid F1 generation, the susceptible and selected strains were reciprocally crossed by mass mating of about 150 virgin adults of each sex. Further datail of genetic analysis will be given in discussion.

Results and Discussion

The adult males and females of the BAN-PR strain were separately submitted to selection with permethrin at a selection pressure of 80-90% mortality. The parental stock (BAN-S) showed an initial KT50 of 39.32 minutes. Selection resulted in a gradual increase in KT50. At the F10 generation, the KT50 reached to its maximun level of 85.02 minutes i.e, 2.2 fold increase in tolerance as compared with the BAN-S strain. The results of permethrin selection are shown in Fig 1. Comparisons between the probit regression lines of the BAN-S and the BAN-PR (F10 generation) indicated that the 40 minutes is a discriminative exposure time for the two strains. In spite of low level of tolerance, attempts were made to study the inheritance of permethrin tolerance. The adults of the BAN-S and the BAN-PR strains were reciprocally crossed and the F1 progeny from the two reciprocal crosses were tested with permethrin. The F1,s showed no significant difference in their responses to permethrin. The results suggest that permethrin tolerance is autosomally inherited. The F1 results also indicated that the observed tolerance is inherited as an intermediate character (see Fig.2).

Piperonyl butoxide (PB), a mixed function oxidase inhibitor was tested in the presence and absence of DDT and permethrin on the BAN-PR and the BAN-S strains. The results are presented in tables 1 and 2. Permethrin in presence and absence of PB on the adult females of the BAN-PR and BAN-S strains showed a synergistic ratio of 1.09 and 1.62 folds respectively, indicating that mixed function oxidase (mfo) plays an important role in the permethrin selected strain and the oxidative detoxification of permethrin by mixed function oxidases might be the major mechanism in the adults of *An.stephensi.* Chlorofenetol (DMC) a dehydrochlorinase inhibitor and PB, a mixed function oxidases were tested in the presence and absence of DDT on the BAN-PR, a DDT resisitant strain. The PB and DMC had no synergistic effect on DDT in DDT resistant strain. The results suggest that probably micorsomal oxidases and dehydrochlorinase are not the important detoxication mechanism in DDT resistant strain. Ladonni (1988) selected a strain of *An.stephensi* from Dubai (DUB-APR) for eight generations with permethrin. Permethrin selection on the adults resulted in an increase in resistance about 10-fold. Crossing experiments suggested that permethrin resistance was inherited as a polyfactorial character with no indication of sex linkage. Following studies on the larvae of Dubai strain (DUB-LPR), showed that this strain is resistant to DDT at the larvae as well as the adult stages. permethrin selection on the larvae resulted in an increase in resistance about 300 fold, compared with the parental stock strain and an enzymatic metabloic pathway was found for observed resistance (4). In an study by Omer et al., (1980), larvae of *An.stephensi* from Pakistan were selected with DDT alone, DDT plus synergists, and permethrin. DDT and permethrin selections resulted in increases in resistance to DDT and permethrin and vice versa. The larval selection also resulted in permethrin resistance at the adult stage. The results suggested that a kdr-type resistant was responsible for the observed resistance to DDT and permethrin.

In this study the cross-resistance/tolerance spectrum of permethrin selected strain was studied to DDT, dieldrin and malathion. The results are shown in table 3. Susceptibility tests on the BAN-S and the BAN-PR strains to DDT 4% for 90 minutes, dieldrin 4% for 50 minutes and malathion 5% for 30 minutes, resulted in 35.29% , 94.87% and 67.57% mortalities for the BAN-S strain respectively and 66.34%, 32% and 47.37% mortalities for the BAN-PR strain respectively. Permethrin selection on the BAN-PR strain decreased susceptibility to dieldrin and malathion but slightly increased susceptibility to DDT. The result suggests that were might be a positive correlation between resistance to malathion, dieldrin and permethrin. Relationship between

resistance to dieldrin and malathion in *An.stephensi* from Pakistan was studied by Rowland in 1985. The genes for dieldrin and malathion was found to be on linkae group III. Ladonni et. at (1992) found a similar correlation between resistance to dieldrin and malathion in *An.stephensi* from Bandar-Abbas. This investigation suggests that there might be a positive correlation between malathion, dieldrin and permethrin resistance in *An.stephensi* from Bandar-Abbas.

Tabel 1- Effect of synergists, DMC and PB in presence and absence of DDT on the susceptible (BAN-S) strain

Synergists/ Insecticide	Dead	alive	%Mortality
DMC PB	0 0	75 76	0 0
DDT	9 9	17 16	35.29
DMC+DDT*	6 10 9 8	12 14 18 14	36.26
PB+DDT*	7 9	15 15	34.78

* The adults were exposed with DDT for 90 minutes following pretreatment with synergists for 90 minutes

Table 2- Effect of Permethrin on adult females of the susceptible (BAN-S) and the selected (BAN-PR) strains in presence and absence of Synergist (PB)

Strains	Insecticide /Synergist	b±SE	KT50±S.E	KT90±S.E	X^2 (d.f)	SR*
BAN-S	PR** PR±PB	9.82±1.19 7.07±0.58	39.32±1.91 36.07±1.47	53.1±4.92 54.75±4.52	7.39(7) 0.96(5)	1.09
BAN-PR	PR PR+PB	11.77±0.98 12.09±0.53	85.02±2.7 52.38±1.55	1109.92±5.52 66.86±3.72	16.81(12) 4.47(5)	1.62

* KT50 of insecticide alone to KT50 of insecticide+PB

** permethrin.

Table 3- DDT, dieldrin and malathion susceptibility tests on adult females of the susceptible (BAN-S) and the selected (BAN-PR) strains

Strains	Insecticides	Exposure time (minutes)	Dead	Alive	%Mortality
BAN-S	DDT	90	34	65	34.34
BAN-PR (F-10)	DDT	90	67	34	66.34
BAN-S	Dieldrin	50	74	4	94.87
BAN-PR (F-10)	Dieldrin	50	32	68	32
BAN-S	Malathion	30	50	24	67.57
BAN-PR (F-10)	Malathion	30	27	35	47.37

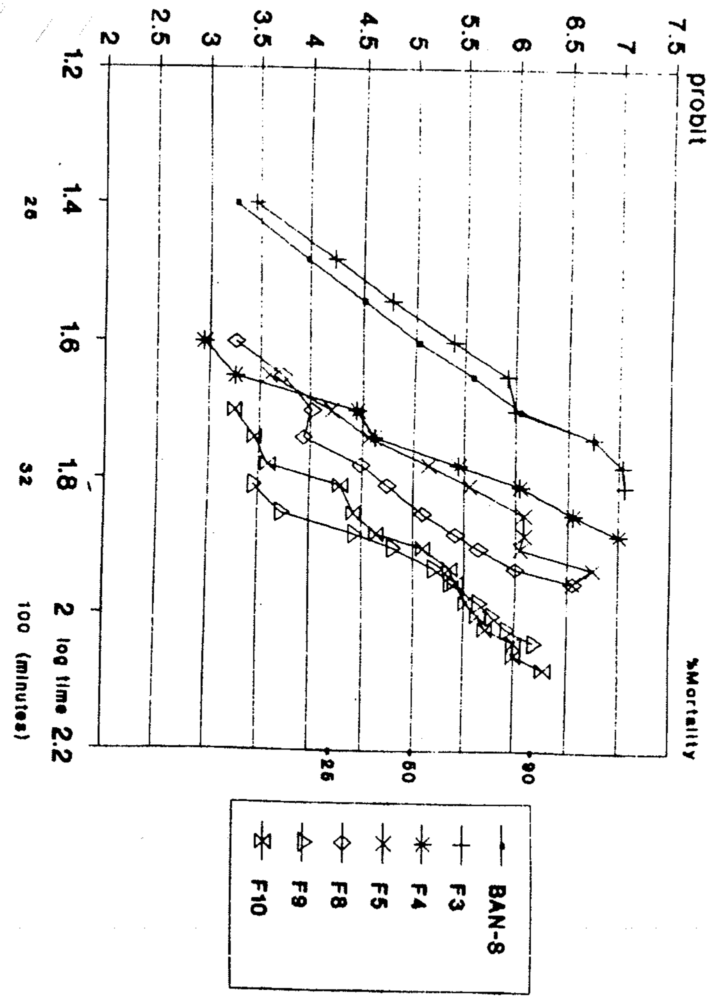

Fig. 1- Log-dose probit regression lines of permethrin selection on BAN-PR strain

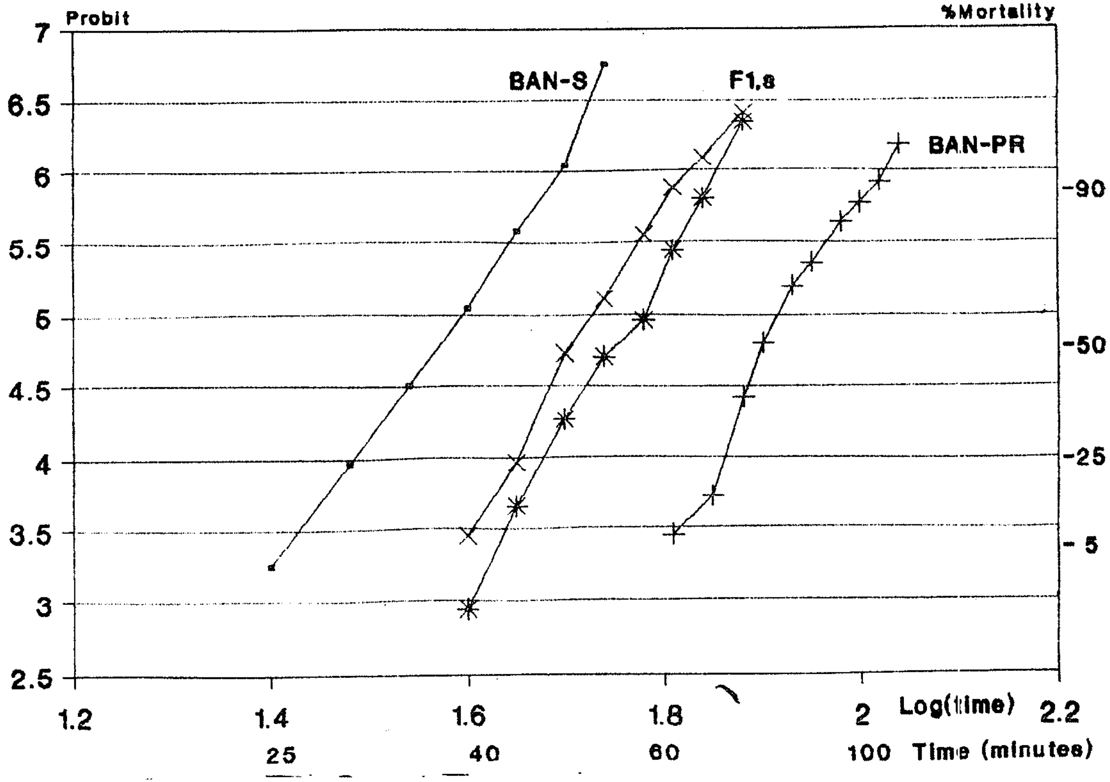

Fig. 2- Probit regression lines of the BAN-S, BAN-PR and F1,s resulted from reciprocal crosses between the two strains

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