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A COMPARATIVE STUDY ON SUSCEPTIBILITY OF THE LARVAE OF TWO WILD STRAINS OF *ANOPHELES STEPHENSI* TO EIGHT INSECTICIDES , IN SOUTH OF IRAN.

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Key words: *Anopheles stephensi , susceptibility , insecticides , Iran*

Abstract

Susceptibility of two wild strains of *An.stephensi* collected from Kazeroun (ST-KAZ and Bandar-Abbas (ST-BAN), south of Iran was compared to fenthion , malathion , temephos , pirimiphos-methyk , deltamethrin , DDT , dimilin and etopenprox as larvicides in the laboratory.

Susceptibility test on the ST-KAZ strain indicated that this strain is susceptible to the named insecticides i.e the resistance ratios remained between 0.91 to 1.37 folds that of the ST-TEH strain , the susceptible laboratory stock .The ST-BAN strain has the long history of insecticide application like , DDT , dieldrin , malathion , propoxur , actellic , lambdacyhalothrin(icon) as adulicides and abate as a larvicide , therefor it showed different pattern of susceptibility to the latter insecticides. The ST-BAN strain showed susceptibility to DDT , deltamethrin , etofenprox and dimilin , moderately tolerant to fenthion and temephos (i.e.resistance ratios ranged between 1.67 to 1.75 folds) and tolerant to pirimiphos-methyl and malathion with resistance ratio of about 2.37 folds , that of the ST-TEH strain. The observed tolerance in the ST-BAN strain to pirimiphos-methyl (actellic) and malathion might be as the result of regular use of adulticides or abate in malaria control programs in south of Iran and develop of cross-tolerance in An.stephensi from Bandar-Abbas, south of Iran.

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Introduction

In south of Iran , *An.stephensi* is one of the main malaria vector. It is known to be resistant to DDT , dieldrin and malathion at the adult stage (7,6,5). After appearance of malathion resistance in *An.stephensi* , propoxur was subsituted in 1978 and it was used for about 13 successive years (two round spraying per year). Subsequently From 1990 to 1992 , the residual spraying was continued with pirimiphos-methyl and propoxur. However , no resistance has been detected in *An.stephensi* to this latter insecticides so far. In recent years pyrethroids are currently receiving con siderable attention as cndidiate chemical for residual spraying in malaria control programmes. Therefore from 1992 lambdacyhalothrin as a pyrethroid was introduced in malaria control progammes. Larval control in south of Iran are now based on chemical control using temephos (50% EC) , natural organic chemicals using petroleum oils and biological control using larvivorous fish (Gambosia affinis)(8).

Resistance to DDT , dieldrin and malathion mainly in the adults of *An.stephensi* , have been widely distributed in Persian-Gulf , Middle-East and Indian-subcontinent areas (1,12,13) , but resistance in the larvae as the result of field application is only limited to DDT, e.g. DDT resistance in Dubai (3) and low level of resistance in Pakistan(9).

In view of the wide spread occupance of resistance to conventional insecticides in anopheline mosquitoes. This is an attempt to study the susceptibility of larvae of two wild strains of *An.stephensi* to a number of currently used/new chemicals and also collect base line data for malaria control programmes in south of Iran.

Materials and methods

The following strains of *An.stephensi* were used in this investigation. ST-TEH strain: A laboratory stock of *An.stephensi* , resistant to DDT and dieldrin at the adult stage but susceptible at the larvae. BAN-S strain: a wild strain based on adults , collected from Chellow village , Bandar - Abbas , costal area of Persian Gulf , south of Iran in April 1991. KAZ-S strain: a wild strain based on adults , collected from Pirsabs village, Kazeroun , south of Iran in August 1991.

The following insecticides were used in this investigation: Pirimiphos-methyl (actellic , 90.7% purity ,ICI Argochemicals). Malathion (95% Purity , Calliope France). Fenthion (96.6% purity , Bayer AG).

PP DDT (75% W.P). Etofenprox (trebon,96.3% purity , Mitsui Toatsu Chemical). Deltamethrin (99.1% purity , Wellcome). Diflubenzuron (dimilin 48% SC. Philip-Duphar , B.V.). Temephos (abate , 50 EC , American Cyanamid).

Mosquito rearing and maintenance was carried out in an insectary at 26-28 C and 70-80% relative humidity , with a 12 hour photoperiod. After initial colonization of the wild strains for three generations , the larvae were tested with insecticides (except dimilin) at the late third instar larvae according to the method described by WHO(11). The larvae were exposed to 4-5 different concentration of insecticide and at each concentration , 100 larvae representing four individual replicates of 25 larvae were tested. In dimilin (48%SC) was diluted in distilled water and 1 ml of this solution was placed in an enameltray containing 2 liters of tap water prior to adding the first instar larvae. The larvae were tested in lots of 150-200 larvae/tray at 4-5 different concentrations (2 replicates per concentration). The larvae were fed with Bemax (baby food) and mortality counts were taken daily during the continuous exposure time. Due to knock down effect of deltamethrin and trebon, the mortality counts, were made after 24 hour exposure followed by 24 hours holding time in fresh water. The pooled results were analyzed by probit analysis (2) , using a statistical package (probit 79) on a personal computer. in that the goodness of fit of the points to a straight line were tested by X^2 analysis and LC50 , LC90 and 95% confidence limits were also estimated for each strain.

Results and discussion

The results of the probit regression line parameters of three strains of *An.stephensi* , tested with 8 commonly used/new insecticides are presented in table. 1. In this study the susceptibility of the ST-KAZ and the ST-BAN , the wild strains of *An.stephensi* were compared with the ST-TEH strain , the laboratory susceptible strain.

Susceptibility tests on the ST-TEH strain with OP compounds such as fenthion, malathion , pirimiphos-methyl and temephos showed the original LC50

of 0.0049+0.00025 , 0.0.0819+0.0115 , 0.0155+0.0018 and 0.0016+0.00034 mg/l respectively. Comparisons of the resistance ratios of ST-KAZ indicated that the ST-KAZ strain has similar pattern of susceptibility to these latter insecticides , i.e. the resistance ratios in the ST-KAZ ranged between 0.91 to 1.37 folds that of the ST-TEH strain (see table 1)in contrast. The ST-BAN strain showed higher resistance ratio than the ST-KAZ strain i.e. the resistance ratios ranged between 1.67 to 2.39 folds that of the ST-TEH strain. The ST-KAZ strain was collected from Kazeroun area , where the insecticide application has been withdraw since 1981. Therefore ST-TEH and the ST-KAZ strains showed similar responses to all insecticides tested. The ST-BAN strain showed 1.75 , 2.37, 2.39 and 1.67 folds increased in tolerance to abate, pirimiphos-methyl , malathion and fenthion respectively. The observed tolerance in the ST-BAN strain could be as the results of long term used of abate as a larvicide and different adulticide e.g. organochloride (DDT , dieldrin) , organophosphorus (malathion and pirimiphos-methyl) and carbamate (propoxur) in malaria control programmes in Bandar-Abbas. The ST-BAN strain showed higher LC50 to pirimiphos-methyl (2.37 fold) and malathion (2.39 fold) than the abate (1.75 fold). The increase tolerance in the ST-BAN might be as the result of temephos selection on the larvae and development of tolerance in the ST-BAN strain to the named insecticides.

DDT test on the ST-KAZ and the ST-BAN strains showed the resistance ratios of 0.91 and 0.99 folds that of the ST-TEH strain respectively , indicating that the two strains are susceptible to DDT at the larval stage. In spite of development of DDT resistance in the adults of the ST-KAZ and the ST-BAN strain (4), the larvae showed susceptibility to DDT.

The result of this study indicating that resistance could not develop larval resistance in the ST-Kaz and the ST-BAN strains.

Etopenprox (trebon) as a new compound with insecticide activity like pryrethroids , deltamethrin as pyrethroid and dimilin as an insect growth regulator (chitin synthesis inhibitor) were also tested against the ST-TEH the St-KAZ and the ST-BAN strains. Susceptibility tests on the larvae indicated that three strains are susceptible to these latter insecticides i.e. the resistance ratios for ST-KAZ and the ST-BAN strains ranged between 1.11 to 1.33 folds that of the ST-TEH strain. The order of toxicity of these

issecticides based on the comparisons of the LC50 of each strain with different insecticide were found to be deltamethrin= temephos= dimilin> trebon> DDT.The susceptibility of *An.culicifacies, An.annulair* and *An.stephensi* to seven insecticides by Rajvanshi et.al.(1981). The order of toxicity were chlorpyrifos temephos pirimiphos-methyl malathion propoxur HCH DDT for *An.stephensi* and *An.culicifacies.* In *An.annulair* the order of toxicity was the same except in case of HCH i.e DDT HCH. Based on the results of this study , the larvae of ST-BAN s train showed susceptibility to dimilin , deltamethrin , DDT (resistance ratios ranged between 0.99 to 1.31 folds) , moderately tolerant to fenthion and abate (resistance ratio ranged between 1.67 to 1.75 folds) and tolerant to pirimiphos-methyl and malathion (resistance ratio ranged between 2.37 to 2.39 folds).

Table 1- Probit regression line parameters of larvae of three strains of *An.stephensi* tested with different insecticides.

Insecticides	Strains	b+SE	LC50+SE	LC90+SE	Resistance ratios
Fenthion	ST-TEH	9.59+1.23	0.0049+0.00025	0.0067+0.0007	-
	ST-KAZ	4.78+0.506	0.0052+0.00075	0.0096+0.0019	1.06
	ST-BAN	6.47+0.608	0.0072+0.00055	0.0114+0.0015	1.67
Malathion	ST-TEH	6.043+0.648	0.0819+0.0154	0.256+0.037	-
	ST=KAZ	12.94+1.428	0.112+0.0115	0.45+0.026	1.37
	ST-BAN	5.34+0.603	0.196+0.049	0.812+0.134	2.39
Pirimiphos-methyl	ST=TEH	4.346+0.464	0.0155+0.0018	0.0305+0.0068	-
	ST-KAZ	4.129+0.314	0.0201+0.0012	0.0532+0.0038	1.3
	ST-BAN	3.96+0.412	0.0367+0.0042	0.0774+0.0151	2.37
DDT	ST-TEH	3.423+0.409	0.546+0.0064	1.129+0.0354	-
	ST-KAZ	2.817+0.227	0.498+0.0078	1.72+0.0129	0.91
	ST-BAN	2.012+0.337	0.539+0.0098	1.34+0.175	0.99
Trebon	ST=TEH	8.0057+1.59	0.0061+0.0015	0.0089+0.0054	-
	ST-KAZ	7.168+1.06	0.0068+0.0028	0.0104+0.0036	1.11
	ST-BAN	6.16+0.554	0.0073+0.005	0.0118+0.0013 5	1.2
Deltamethrin	ST-TEH	2.98+0.284	0.0012+0.00015	0.0033+0.0008 5	-
	ST-KAZ	3.48+0.184	0.0016+0.00031	0.0041+0.0001 2	1.33
	ST-BAN	2.34+0.296	0.0015+0.00035	0.0052+0.0028 5	1.25
Temephos	ST=TEH	3.51+0.237	0.0016+0.00034	0.0032+0.0002 8	-
	ST-KAZ	2.98+0.467	0.0019+0.00033	0.0041+0.0006 5	1.19
	ST-BAN	3.354+0.713	0.0028+0.00071	0.0053+0.0003 9	1.75
Dimilin	ST-TEH	1.102+0.85	0.0026+0.00024	0.0046+0.0002 3	-
	ST=KAZ	0.884+0.12	0.003+0.00067	0.0091+0.0005 8	1.15
	ST-BAN	1.079+0.093	0.0034+0.00051	0.0055+0.0001 6	1.31

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