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Irritability Level of *Anopheles Stephensi* to Different Insecticides in Iran

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ABSTRACT

Susceptibility levels of a lab strain of *An. Stephensi* to WHO standard papers of DDT 4%, dieldrin 0.4%, malathion 5%, permethrin 0.25%, lambdacyhalothrin 0.1%, cyfluthrin 0.1% and deltamethrin 0.025% were determined in our laboratory in 1999. Results showed that at the diagnostic dose of insecticides this species exhibited resistance to DDT and dieldrin with mortality rate of 54±2.5% and 30±2.5%, respectively. Malathion 5% caused 96±1% mortality. Permethrin killed 90±2% of the populations. Alpha-cyano groups of pyrethroids, e.g., deltamethrin, cyfluthrin and lambdacyhalothrin had highest efficacy, the mortality was 99.5±0.5, 100%, and 99±0.5% respectively. The results of irritability of this species to lambdacyhalothrin, permethrin cyfluthrin and deltamethrin revealed that lambdacyhalothrin had the most and deltamethrin the least irritancy effect. The average number of take offs/fly/minutes for lambdacyhalothrin and deltamethrin were 1.699±0.35 and 0.946±0.13%, respectively. For permethrin and cyfluthrin the equivalent values were 1.52±0.2 and 1.385±0.25, respectively.

INTRODUCTION

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A number of vector-borne diseases are of great importance in Iran including malaria and leishmaniosis. Of these malaria is the most important in terms of mortality and morbidity. Out of 19 Anopheles species in Iran only eight of them play a role in malaria transmission. *Anopheles stephensi, An. culicifacies and An. Fluviatilis* are the major malaria vectors in malarious areas in southern part of Iran. According to National Malaria Control Strategy, the control of mosquito vectors is directed either toward the immature stages or adults. There are different methods for control of vectors including environmental management, chemical, and biological control. Chemical control through the use of insecticides has been contributed enormously to vector control and hence the control of mosquito-borne infections in the world. With the emergence of resistance to organochlorine, organophosphates and carbamates insecticides as well as environmental hazards of this products, attention was focused on pyrethroid insecticides. Currently pyrethroid insecticides represent important weapons against pests of both economic and medical importance. Numerous projects are now in progress using pyrethroids as residual application and impregnated bednets in many parts of the world (8).

The rational use of insecticides largely depens on a broad knowledge of the susceptibility and irritability levels of malaria vectors to currently used insecticides. In This study we will investigate the irritability level of laboratory reared *Anopheles stephensi* originated form Iran to different insecticides which are currently used for mosquitoe control in Iran. This knowledge enables us to take all necessary precautions to prevent the occurrence of resistance and to prepare in advance a plan for coping with it at the early stages of its development in the field.

MATERIALS AND METHODS

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Insecticide Impregnated Papers

The following insecticide impregnated papers provided by WHO were used; DDT 4%, dieldrin 0.4%, malathion 5%, lambdacyhalothrin 0.1%, permethrin 0.25%, cyfluthrin 0.1% and delta-methrin 0.25%.

Insecticide Testing Method

Tests on adults were carried out according to the methods of WHO (10). Female mosquitoes were exposed at the diagnostic dose of insecticides for one hour. To reduce the variability in the replicates, 2-3 day old sugar fed adults were used. At each exposure time 25 adults were tested. Depend on availability of mosquitoes and insecticides 52-1017 mosquitoes were tested representing 2-40 individual replicates of adults. Due to knock-down effect of pyrethroids on the adutls, the exposure tubes were held in a horizontal position during the tests. The mortality was scored after a 24 hour recovery period. Insecticide exposures took place with a temperature of 24-28°C and holding tubes were held in a insectary under controlled conditions of 25±1°C and 60-70% relative humidity.

Irritability Testing Method to Pyrethroids

The level of irritability of mosquitoes was measured according to the method described by WHO (9). 20 unfed 2-3 day old females of strain of *An. Stephensi* were individually exposed to the diagnostic dose of pyrethroids (0.25% permethrin, 0.1% cyfluthrin, 0.025% deltamethrin and 0.1% lambdacyhalothrin) in an exposure chamber and the number of take offs were counted during a 15 minutes exposure time. The mean and standard deviation of number of take-offs for individuals were calculated. The irritability of *An. Stephensi* to different pyrethroids were plotted and determined by analysis of variance.

RESULTS AND DISCUSSION

Susceptibility Levels of *An. Stephensi* to Different Insecticides

Adult susceptibility test on this species using WHO impregnated papers revealed that adult females were resistant to organochlorine insecticides such as DDT and Dieldrin. Mortality rate to these insecticides was 54±2.5% and 30±2.5% respectively. DDT resistance in *An. Stephensi* as tested in different countries such as Iraq in 1957 (3), Saudi Arabia in 1955 (2), Iran in 1957 (6), and India in 1965 (4). Dieldrin resistance was first reported from Iran after 1-2 year starting application in 1959 (11). Malathion exhibited 96±1% mortality which shows a high susceptibility to this insecticide. In 1974 Manouchehri et al. reported that a field population of *An. Stephensi* in Iran was resistant to DDT and dieldrin but susceptible to malathion. Subsequently they reported the development of malathion resistance in the population originated from Bandar Abbas, Iran (5). To find out base line susceptibility of laboratory stains of *An. Stephensi* to pyrethroids impregnated paper the mosquitoes were exposed at different interval times. To plot the log-probit regression lines between mortality and exposure time the diagnostic pyrethroids gave 100 percent knock-down after posure. Therefore, due to highly exhibition of this strain to pyrethroids effort was made to f *An. Stephensi* at the diagnostic dose. The exposed one hour to these insecticides and covery period, the mortality were scored. The experiments were tested at different occasions in different replicates. The results are presented in Table 1. In our study new generations of pyrethroids with alpha cyano groups, e.g., deltamethrin, cyfluthrin and lambdacyhalothrin exhibited highest efficacy against this species. The mortality rates for these insecticides were 99.5±0.5%, 100% and 99±0.5% respectively (Table 1 and Fig.1). Among pyrethroids only permethrin showed 90±2% mortality. DDT and permethrin cross-resistance was reported in *An. stephensi* (7). They suggested *kdr* play a major role in the resistance. In the work of Charkrovorthy & Kalyanasundaram in 1992 (1) adults of *An. stephensi* were selected with permethrin in the laboratory in India. The selection resulted the development of resistance of 13-fold to permethrin, and cross-resistance to 7-fold to cypermethrin and 10-fold to deltamethrin. The development of cross-resistance to 4% DDT was also detected. In our study although further experiments are needed to find the functional basis of DDT resistance in the laboratory population, but evidence showed that there was no cross-resistance between organochlorine used and new generation of pyrethroids. Further filed and laboratory studies are required to clarify the involvement of *Kdr* gene in DDT and permethrin resistance in this strain.

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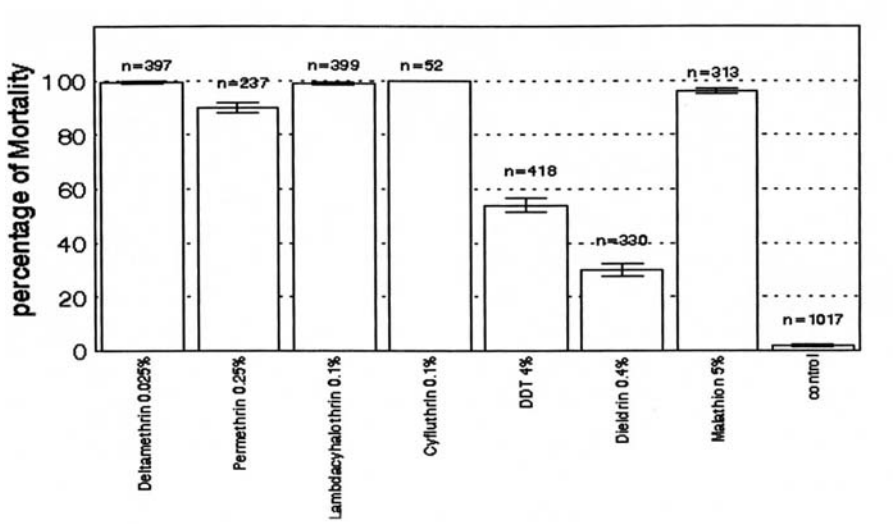
Fig. 1. **The mortality of *An. Stephensi* females exposed to a diagnostic dose of insecticides**



Irritability Level of *An. stephensi* to Pyrethroids

The irritant property of some insecticides can cause a proportion of insects to leave sprayed surfaces before acquiring a lethal dose, so the repeated contact is required before mortality occurs. The term repellency (more often excito-repellency) is sometimes related to this phenomenon. Repellency is the prevention of the insect from approaching the insecticide. This irritability would produce heightened activity in the landing mosquito and will only remain on the treated surface for a short period of time. The irritability response of vectors were interpreted to have a negative impact on control efforts. Insecticide repellency could prevent vectors from entering human habitations treated with the insecticides. In the long run this is likely to cause reduction in endophilic mosquitoes and an increase in the exophilic populations. Pyrethroids may repel insects due to air-borne repellency or contact, which raise the possibility the behavioural response might be important attributes of pyrethroid use. In some cases survival of a species in the treated houses is attributed to the reduce intrinsic toxicity of insecticide or occurrence of physiological resistance but these phenomenon might be due to irritancy property of insecticide. The irritability to insecticides may reduce the effectiveness of residual applications of the insecticides.

Careful monitoring of both physiological and behavioural responses to pyrethroids will be essential in the evaluating the pyrethroids. In this study the level of irritability of mosquitoes was determined. The results of Irritability of deltamethrin, permethrin, lambdacyhalothrin and cyfluthrin at the diagnostic dose are presented in Table 1 and shown in Fig. 2 and Fig. 3. From this Figures it can be concluded that lambda-cyhalothrin had the most and deltamethrin less irritancy effect against *An. stephensi*. Cyfluthrin exhibited moderate effect. Average number of take offs/female/minute for lambda-cyhalothrin, permethrin, cyfluthrin and deltamethrin was 1.699±0.35, 1.52±0.20, 1.385±0.25 and 0.946±0.13, respectively.

Table 1. **The mortality of *An.stephensi* females exposed to a diagnostic dose of insecticides**

Insecticide	Replicates	No. mosquito tested	No. dead	Mortality (%)	Error bar

Permethrin 0.25%	12	237	213	90	±2.0
Lambdacyhalothrin 0.1%	14	399	396	99	±0.5
Cyfluthrin 0.1%	2	52	100	100	±0.0
Deltamethrin 0.025%	18	397	395	99.5	±0.5
DDT 4%	16	418	226	54	±2.5
Dieldrin 0.4%	14	330	99	30	±2.5
Malathion 5%	14	313	301	96	±1.0
Control	40	1017	20	2	±0.5

Table 2. **Irritability level of *An.stephensi* females to different pyrethroids**

	Lambdacyhalothin 0.1% (n=40)			**Permethrin 0.25% (n=40)**			**Cyfluthrin 0.1% (n=40)**			**Deltamethrin 0.025% (n=60)**		
Time (min)	**No. Take off**	**Mean**	**SE**	**No. Take off**	**Mean**	**SE**	**No. Take off**	**Mean**	**SE**	**No. Take off**	**Mean**	**SE**
1	19	0.47	0.18	15	0.37	0.21	9	0.22	0.11	18	0.30	0.12
2	27	0.67	0.21	17	0.42	0.16	10	0.25	0.13	17	0.28	0.15
3	70	1.75	0.6	41	1.02	0.31	47	1.17	0.38	34	0.56	0.22
4	134	3.35	0.68	95	2.37	0.69	91	2.27	0.5	81	1.35	0.50
5	157	3.92	0.44	95	2.37	0.57	108	2.70	0.62	104	1.73	0.73
6	139	3.47	0.6	98	2.45	0.57	98	2.45	0.55	74	1.23	0.35
7	114	2.85	0.46	98	2.45	0.67	107	3.40	0.70	97	1.6	0.39
8	110	2.75	0.65	95	2.37	0.60	98	2.45	0.55	92	1.53	0.38
9	80	2.00	0.52	60	1.50	0.40	81	2.02	0.56	78	1.30	0.43
10	42	1.05	0.27	70	1.75	0.56	52	1.30	0.38	73	1.21	0.30
11	43	1.07	0.56	67	1.67	0.59	40	1.00	0.34	41	0.68	0.18
12	26	0.65	0.46	53	1.32	0.53	39	0.97	0.46	38	0.63	0.20
13	18	0.45	0.40	42	1.05	0.31	25	0.62	0.28	29	0.48	0.23
14	15	0.37	0.34	36	0.90	0.32	15	0.37	0.19	39	0.65	0.22
15	7	0.17	0.32	26	0.65	0.32	10	0.25	0.13	37	0.61	0.50
Average		1.699	0.34		1.52	0.20		1.385	025		0.946	0.13

Fig. 2. **Irritability level of*An. Stephensi* females to different pyrethroids**

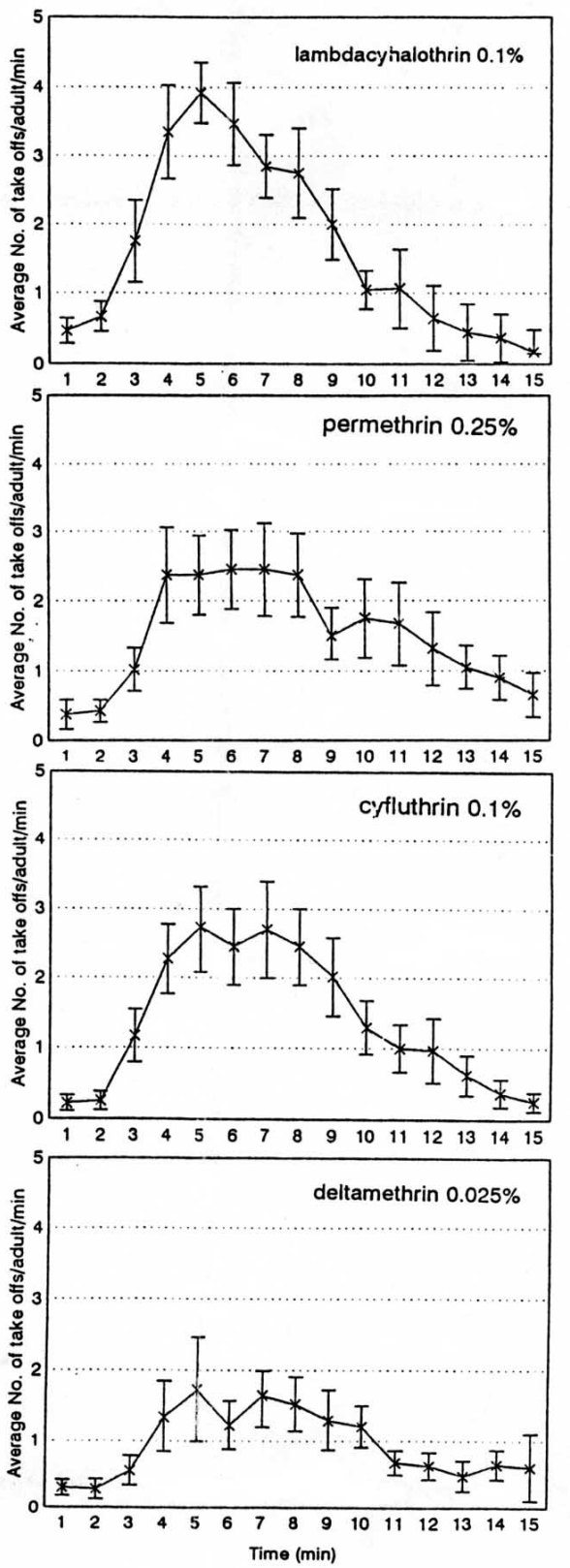
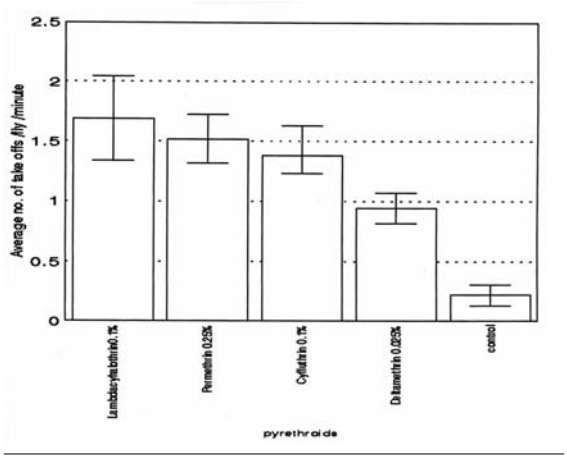


Fig. 3. **Average number of take offs/fly/minutes of *An. Stephensi* to a diagnostic doses of pyrethroids in 15 minutes exposure time.**



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