SUSCEPTIBILITY AND IRRITABILITY LEVELS OF MAIN MALARIA VECTORS TO SYNTHETIC PYRETHROIDS IN THE ENDEMIC AREAS OF IRAN

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Abstract- The rational use of insecticides largely depends on a broad knowledge of the susceptibility and irritability levels of malaria vectors to currently used insecticides especially pyrethroids. In this study the susceptibility and irritability levels of *Anopheles stephensi* and *An.culicifacies* to DDT 4%, malathion 5%, propoxur 0.1%, deltamethrin 0.025%, lambdacyhalothrin 0.1%, cyfluthrin 0.1% and permethrin 0.25% were determined. Susceptibility and irritability tests on adult mosquitoes were carried out according to WHO methods. The results showed that *An.stephensi* was resistant to DDT 4% and mortality rates to this insecticide in Gavdary and Abtar areas were 64.2%±3.9 and 61.8%±4.36, respectively. *An.stephensi* was assumed susceptible to other insecticides. *An.culicifacies* was found susceptible to all the tested insecticides. The irritability tests carried out with pyrethroids exhibited that permethrin 0.25% had the highest irritancy effect against both species. Lambdacyhalothrin 0.1% and deltamethrin 0.025% had the least irritancy effect against *An.stephensi* and *An.culicifacies,* respectively. Average numbers of take offs/females/minute of *An.stephensi* to permethrin, deltamethrin, cyfluthrin and lambdacyhalothrin were 6.64±1.04, 3.11±0.67, 2.73±0.61 and 2.57±0.67, respectively. These figures for *An.culicifacies* were 2.24±0.37, 1.44±0.38, 1.59±0.35 and 1.46±0.5, respectively. Irritancy effect of pyrethroids should come in consideration while they are used for control of malaria vectors.
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Key words: Pyrethroids, *Anopheles stephensi*, susceptibility, irritability

INTRODUCTION

Malaria is considered one of the three most important diseases throughout the world. It is an indicator of poverty and social injustice and a breaking factor in the socioeconomic development. Control of malaria has been encountered with many problems such as insecticide resistance in vectors and drug resistance in parasites. There are two main kinds of insecticide resistance, physiological and behavioral. At the beginning of insecticide use, vectors are not able to resist the toxic effects of the insecticide but they become slowly resistant to the chemicals. Also, continuous use of insecticide may cause exophilicity in the population of mosquitoes in long term. This can take place for several reasons. Vectors may change their indoor resting to outdoor resting habits because of the irritant and repellent action of some insecticides. The mosquitoes can avoid contact with the insecticide by a natural tendency to rest outside houses (exophily) and the irritant property of some insecticides can cause irritation of insects by contact with the insecticides. Mosquitoes may only absorb a sub lethal dose of insecticide when the insecticide formulation causes a locomotor-stimulant effect on mosquitoes.

The level of irritability of mosquitoes to insecticides is subdivided into three classes:

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hyperirritable, moderately irritable and hypoirritable. It is thought that resistant and laboratory strains of mosquitoes are less irritable than susceptible and field strains to some insecticides; however, this is not always true. In some anophelines species, the proportion that escapes the insecticidal action due to irritability can be high, limiting the effectiveness of indoor spraying with insecticides (1). In a recent study carried out by Hougard *et al.*, resistant strains of *Anopheles gambiae* and *Culex quinquefasciatus* had different response of irritability to pyrethroids (2).

The rational use of insecticides largely depends on a broad knowledge of the susceptibility and irritability levels of malaria vectors to currently used insecticides especially pyrethroids. 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. *Anopheles stephensi* and *Anopheles culicifacies* are the major malaria vectors in malarious area in East-southern part of Iran. This study was carried out to determine the susceptibility and irritability levels of *An.stephensi* and *An.culicifacies* to different insecticides in Sistan and Baluchistan province of Iran during the year 2000.

MATERIALS AND METHODS

These strains were collected from different larval breeding places around Iranshahr and transferred to the insectary, where they were maintained and fed with Bemax. The following insecticides impregnated papers provided by WHO were used: DDT 4%, malathion 5%, propoxur 0.1%, lambdacyhalothrin 0.1%, permethrin 0.25%, cyfluthrin 0.1 % and deltamethrin 0.025%. Susceptibility and irritability tests were conducted according to the WHO methods (3).

Female mosquitoes were exposed at the diagnostic dose of insecticides for one hour and then followed for 24 hours recovery period for mortality count.

To determine susceptibility level in *An.stephensi,* 2-3 day old, sugar fed adults were used. *An.culicifacies* were collected as blood-fed females from pit-shelters, indoor dwelling with hand-catch in early morning and biting collections on human and animal baits every night in Ghassreghand district, Sistan and Baluchistan province. At each exposure time 22±3 adults were tested. Due to knockdown effect of pyrethroids on the adults, the exposure tubes were held in horizontal position during the tests. The mortality was scored after 24h recovery period. Insecticides exposure was carried out in temperature of 24-29 °C and holding tubes were held in a insectary under controlled conditions of 25±1°C and 60-80% relative humidity.

The level of irritability of mosquitoes was measured according to the method described by WHO (4).

Sixty unfed 2-3 days old females of field strains of *An.stephensi* and 70 unfed 2-3 days old females of field strains of *An.culicifacies* were individually exposed to the diagnostic dose of pyrethroids (permethrin 0.25%, cyfluthrin 0.1%, lambdacyhalothrin 0.1% and deltamethrin 0.025%) in an exposure chamber. Unfed females were used as a control. The number of take offs was counted during 15 minutes exposure time. The mean and standard deviation of number of take offs for individuals per minute were calculated. The irritability of *An.stephensi* and *An.culicifacies* to different pyrethroids was plotted and determined by analysis of variance. The irritability tests were carried out in a temperature of 24-29°C and 45-55% relative humidity.

RESULTS

The results of susceptibility tests of adults of *An.stephesni* and *An.culicifacies* are given in table 1 and illustrated in figures 1, 2 and 3.

In order to determine the level of irritability of *An.stephensi* and *An.culicifacies* against different pyrethroid insecticides, the average number of take offs in a standard given time of 15 minutes were compared. The results of irritability level of *An.stephnsi* and *An.culicifacies* to deltamethrin 0.025%, permethrin 0.25%, cyfluthrin 0.1 % and lambdacyhalothrin 0.1% at the diagnostic dose are presented in tables 2 and 3 and shown in figures 4 and 5.

Table 1. The susceptibility level of field strain of *Anopheles stephesni* and *Anopheles culicifacies* to different insecticides, Abtar, Gavdary and Ghassreghand villages, Iranshahr, Sistan and Baluchistan province, Iran, 2000

	Anopheles stephesni				*Anopheles culicifacies*	
	Gavdary		Abtar		Ghassreghand	
Insecticides	**No. tested**	**Mortality rate ±SE**	**No. tested**	**Mortality rate ± SE**	**No. tested**	**Mortality rate ±SE**
Malathion 5%	119	96.8±1.5	103	99±0.9	102	100%
DDT 4%	117	64.2±3.9	110	61.8±4.63	125	99.1%± 0.84
Permethrin 0.25%	75	97±1.36	-	-	92	100%
Bendiocarb 0.1%	75	98.5±1.4	-	-	-	-
Lambdacyhalothrin 0.1%	109	96.7±1.78	95	100±0	85	100 %
Propoxur 0.1%	138	98.5±1.4	97	98.9±1.05	93	96.5%±1.9
Deltamethrin 0.025%	100	96.6±1.7	87	100±0	107	100 %
Control	212	6.6±1.7	101	0±0	232	5.78%±1

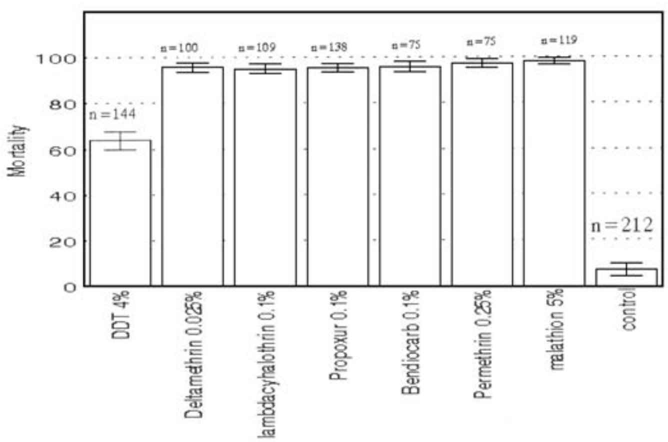

Fig. 1. Susceptibility level of *An.stephensi* to different insecticides in Gavdary, Iranshahr, Sistan and Baluchistan province, Iran, 2000.

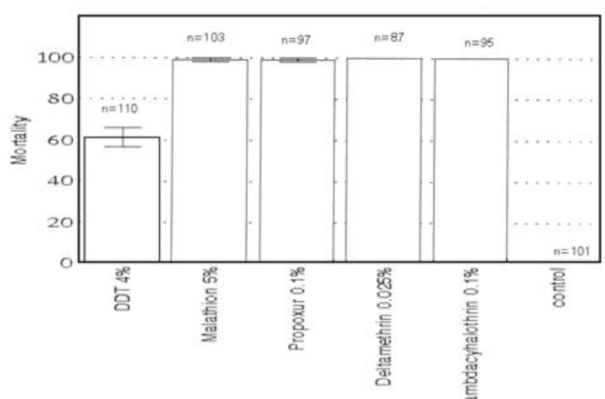

Fig. 2. Susceptibility level of *An.stephensi* to different insecticides in Abtar, Iranshahr, Sistan and Baluchistan province, Iran, 2000.

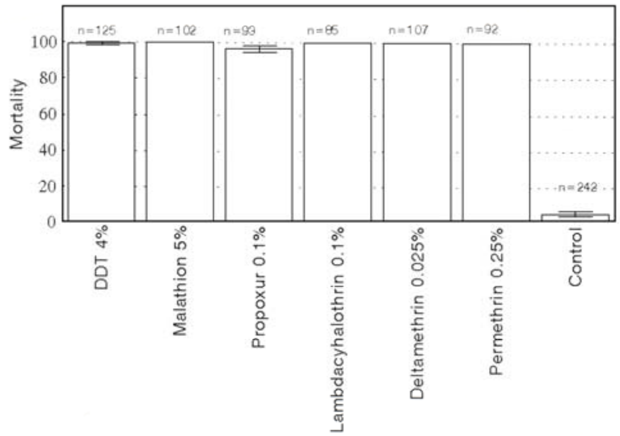

Fig. 3. Susceptibility level of *An.culicifacies* to different insecticides in Ghasreghand district, Iranshahr Sistan and Baluchistan province, Iran, 2000.

Table 2. The average number of take offs /minute/females of field strain *of An.stephensi* to different pyrethroids in Iranshahr, Sistan and Baluchistan province, Iran, 2000

Time	Lambdacyhalothrin 0.1% (n=60)	Cyfluthrin 0.1% (n=60)	Deltamethrin 0.025% (n=60)	Permethrin 0.25% (n=60)	Control (n=40)
1	1.00(0.15)	1.56(0.21)	1.51(0.35)	2.6(0.39)	0.25(0.12)
2	6.85(0.55)	5.36(0.57)	5.51(0.64)	9.44(1.03)	0.30(0.14)
3	8.68(0.60)	8.58(0.60)	8.63(0.71)	13.45(0.92)	0.27(0.09)
4	5.8(0.53)	6.21(0.55)	7.36(0.61)	12.59(0.88)	0.52(0.39)
5	4.73(0.57)	5.06(0.50)	6.26(0.61)	12.36(1.01)	0.35(0.28)
6	2.41(0.35)	2.88(0.43)	4.25(0.52)	9.95(0.90)	0.22(0.09)
7	2.10(0.34)	1.98(0.32)	2.85(0.50)	8.59(0.79)	0.15(0.12)
8	1.13(0.22)	1.91(0.30)	1.88(0.33)	6.5(0.71)	0.15(0.10)
9	1.26(0.24)	1.43(0.30)	1.33(0.26)	4.54(0.55)	0.17(0.0)
10	0.96(0.20)	1.15(0.27)	1.16(0.26)	4.08(0.57)	0.17(0.09)
11	0.85(0.18)	1.36(0.20)	1.13(0.22)	3.47(0.56)	0.15(0.05)
12	1.00(0.20)	0.76(0.18)	1.28(0.28)	3.49(0.50)	0.07(0.05)
13	0.70(0.17)	0.98(0.16)	1.33(0.24)	3.39(0.49)	0.07(0.05)
14	0.70(0.16)	0.91(0.16)	0.9(0.17)	2.98(0.50)	0.05(0.05)
15	0.51(0.16)	0.83(0.17)	1.28(0.32)	2.26(0.37)	0.00(0.00)
Mean	2.57(0.67)	2.73(0.61)	3.11(0.67)	6.64(1.04)	0.20(0.13)

*Data are given as mean (SE).

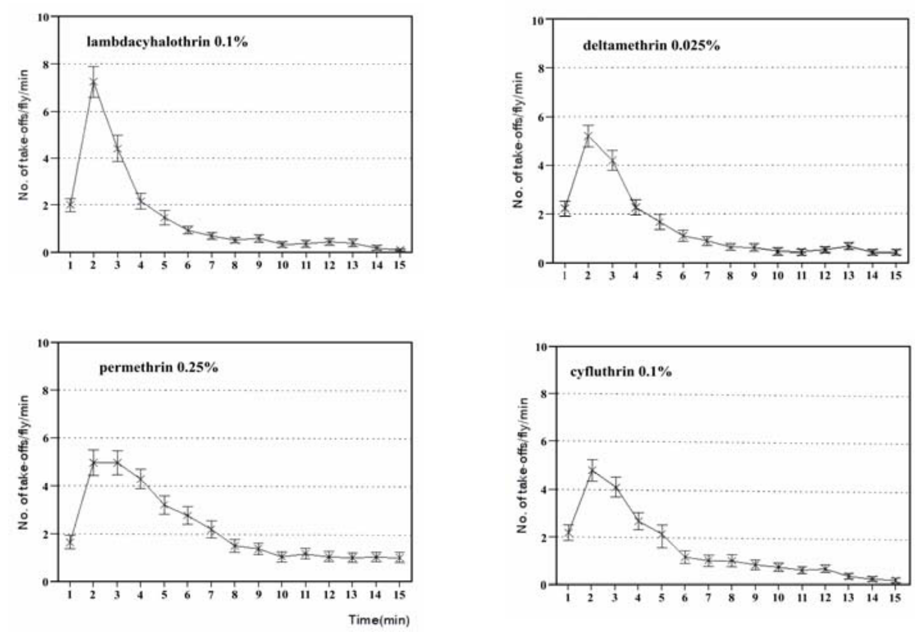

Fig. 4. Irritability level of field strain of *An.stephensi* to different pyrethroids in Iranshar, Sistan and Baluchistan province, Iran in 2000.

Table 3. The average number of take offs /minute/ females of field strian of *An.culicifacies* to different pyrethroids. Iranshahr, Sistan and Baluchistan province Iran, 2000*

Time	Lambdacyhalothrin 0.1% (n=70)	Cyfluthin 0.1% (n=70)	Deltamethrin 0.025% (n=70)	Permethrin 0.25% (n=70)	Control (n=40)
1	2.00(0.28)	2.18(0.31)	2.24(0.29)	1.66(0.28)	0.22(0.05)
2	7.24(0.66)	4.80(0.45)	5.21(0.43)	4.98(0.53)	0.25(0.12)
3	4.40(0.57)	4.10(0.43)	4.21(0.42)	4.98(0.5)	0.22(0.09)
4	2.16(0.33)	2.68(0.36)	2.27(0.33)	4.30(0.42)	0.27(0.12)
5	1.47(0.30)	2.15(0.39)	1.67(0.31)	3.22(0.38)	0.3(0.08)
6	0.93(0.16)	1.20(0.27)	1.10(0.24)	2.78(0.36)	0.35(0.12)
7	0.62(0.14)	1.06(0.24)	0.88(0.19)	2.21(0.36)	0.22(0.17)
8	0.52(0.13)	1.06(0.26)	0.61(0.15)	1.54(0.26)	0.17(0.09)
9	0.60(0.16)	0.90(0.22)	0.60(0.16)	1.42(0.23)	0.05(0.05)
10	0.35(0.12)	0.81(0.19)	0.45(0.15)	1.09(0.22)	0.05(0.05)
11	0.38(0.15)	0.70(0.15)	0.42(0.11)	1.22(0.22)	0.02(0.15)
12	0.46(0.14)	0.76(0.17)	0.50(0.11)	1.10(0.22)	0.05(0.05)
13	0.41(0.16)	0.45(0.12)	0.64(0.15)	1.06(0.21)	0.00(0.00)
14	0.21(0.10)	0.35(0.11)	0.40(0.10)	1.09(0.21)	0.00(0.00)
15	0.12(0.05)	0.31(0.09)	0.40(0.10)	1.07(0.22)	0.00(0.00)
Mean	1.46(0.50)	1.56(0.35)	1.44(0.38)	2.24(0.37)	0.15(0.12)

*Data are given as mean (SE).

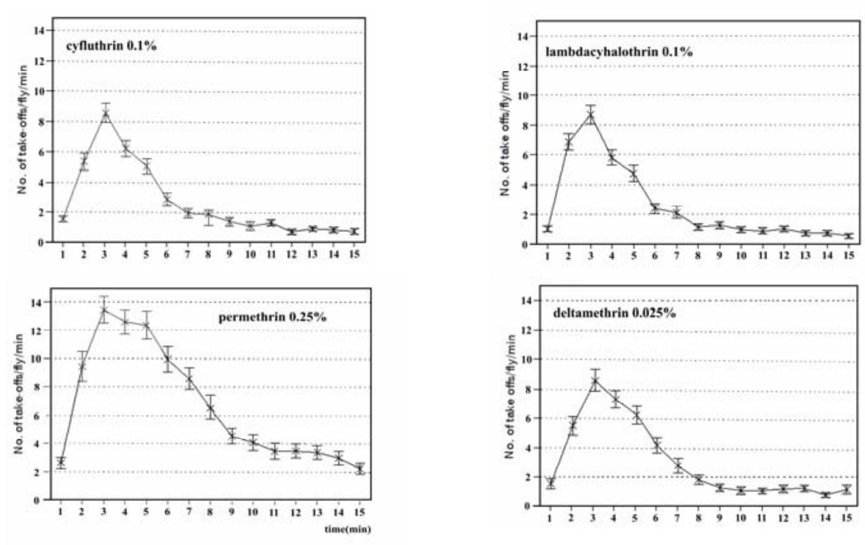

Fig. 5. Irritability level of field strain of *An.culicifacies* to different pyrethroids in Iranshar, Sistan and Baluchistan province, Iran, 2000.

DISCUSSION

Results revealed that *An.stephensi* was resistant to DDT 4% in both tested areas (Gavdary and Abtar); mortality rate to DDT 4% ranged between 64.2±3.9% and 61.8±4.36%, respectively. DDT resistance in *An.stephensi* was reported in 1957 from Iraq (5), in 1951 from Saudi Arabia (6), in 1957 from Iran (7) and in 1965 from India (8). *An.stephensi* was assumed to be susceptible to other insecticides in both tested areas. The adults of *An.culicifacies* were found susceptible to all insecticides tested including DDT 4% (Table 1, Fig 3). From the figures of irritability, it can be concluded that permethrin had the highest irritancy effect against both *Anopheles* species. Lambdacyhalothrin and deltamethrin had the least irritancy effect against *An.stephensi* and *An.culicifacies*, respectively. By using one way analysis of variance (ANOVA) results showed that there was a significant difference among different pyrethoids (F=0.00 for *An.stephensi* and F=0.0033 for *An.culicifacies*).

Statistical analysis (LSD and Duncan tests) showed that permethrin induced significantly more take offs than all other tested materials against *An.stephensi*; there was not any significant difference among deltamethrin, cyfluthrin and lambdacyhalothrin. Statistical analysis in case of *An.culicifacies* showed no significant difference in number of take offs induced by four tested pyrethroids (deltamethrin, permethrin, cyfluthrin and lambdacyhalothrin). The irritability level of *An.culicifacies* in comparison with *An.stephensi* against four pyrethroids was low (Tables 2 and 3, Figures 6 and 7).

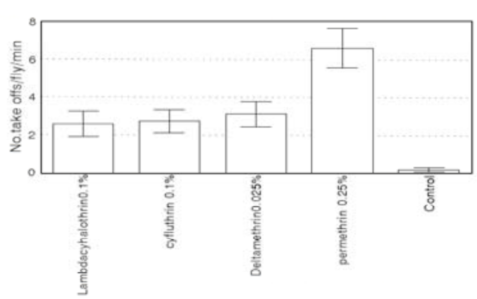

Fig. 6. Average number of take offs/adult/minute to different pyrethroids in *An.stephensi*.

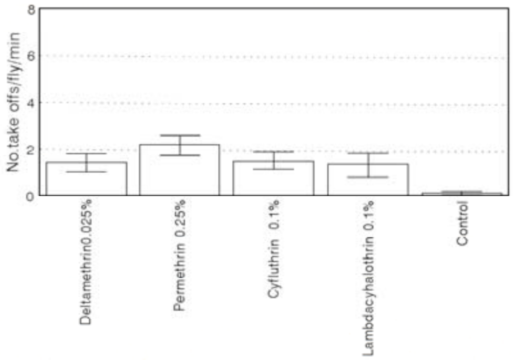

Fig. 7. Average number of take offs/adult/minute to different pyrethroids in *An.culicifacies*.

Behavioral resistance appears more rapidly in endophilic species than exophilic ones. *An.stephensi* has more endophilicity habit than *An.culicifacies* (9), so it is assumed that this species has been selected under insecticides pressure. Irritability and susceptibility levels of endophilic *An.superpictus* and exophilic *An.hyrcanus* to malathion, fenitrothion, propoxur and DDT with WHO insecticide impregnated papers have been determined previously (10). The results showed that *An.superpictus* was completely susceptible to malathion, fenitrothion and propoxur, had a low resistant to DDT and was hyper to moderately irritable to this insecticides. On the contrary, *An.hyrcanus* had a low irritability to insecticides but high resistance to DDT and moderate resistance to propoxur, while its susceptibility to malathion and fenithrothion remained complete. It is supposed that the nature of adaptation to insecticide pressure is, to a great extent, determined by mosquito endophily or exophily. Similar tests in other countries have shown that *An.culicifacies* is more susceptible and has a lower irritability than *An.stephensi* to DDT (11). In both Anopheles species tested in this study, permethrin 0.25% had the most irritability effect. Average number of take offs/adult/min in *An.stephensi* and *An.culicifacies* were 6.64±1.04 and 2.24±0.37, respectively. Vapor pressure of permethrin (0.001 mpa) is lower than deltamethrin and lambdacyhalothrin (0.002 mpa), so the atmosphere within the test chamber would be quickly saturated with vapor, leading to rapid habituation of the olfactory organs.

Rutledge *et al.* found that permethrin had more irritancy effect than conventional repellents on *Aedes aegypti* and *Ae.taeniorhynchus* (12). Permethrin affects tarsal organs and its repellency effect or vapor pressure is lower than conventional repellents. On the other hand, conventional repellents affect antennal organs, and the atmosphere within the test chamber would be quickly saturated with repellent vapor, leading to rapid habituation of the olfactory or antennal organs. Vatandoost found that resistant strain of *An.stephensi* had less irritability to permethrin than susceptible ones (13). In another study carried out by Vatandoost, laboratory strain of *An.stephensi* exhibited different susceptibility to pyrethroids (14).

In conclusion, this study expresses that the irritancy effect of pyrethroids should come in consideration while they are used for control of malaria vector as a residual insecticides as well as impregnated bed nets. Change of behavior of vectors seems a to be beneficial for control of malaria because of reduction of contact of human in short term, but it is harmful in long term, because it will cause change of behavior of mosquitoes and increase in exophilic populations.

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