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Comparative Efficacy of Different Imagicides Against Different Strains of *Anopheles stephensi* in the Malarious Areas of Iran, 2004-2005

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Abstract: Using of insecticides depend largely on the knowledge of the susceptibility levels of malaria vectors to these chemical. In this study, the susceptibility levels of *Anopheles stephensi* to DDT 4%, dieldrin 0.4%, fipronil 1%, deltamethrin 0.05%, permethrin 0.75%, lambdacyhalothrin 0.05% and cyfluthrin 0.15% were determined according to WHO methods in three endemic malarious regions; Sistan and Baluchistan, Hormozgan and Fars provinces. The LT_{50} values of different insecticides were calculated using the probit regression line for each strain. The results showed that *An. stephensi* is resistant to DDT, dieldrin and fipronil in all regions; however Bandar Abbas strain showed the highest level of resistance to these three insecticides. All the tested strains were sensitive against four pyrethroid insecticides. The LT_{50} of Bandar Abbas strain against cyfluthrin and deltamethrin with 5.46 and 3.22 min were the highest and the lowest values respectively. The highest and the lowest mortality rates of the Kazeroon strain of *An. stephensi* were against lambdacyhalothrin and permethrin with 6.80 and 0.682 min, respectively. In Iranshahr region, the figures for deltamethrin, permethrin, lambdacyhalothrin and cyfluthrin were 3.50, 5.10, 3.95 and 5.38 min, respectively. The results of the study suggested a possible cross-resistance between dieldrin and fipronil. Regarding the sensitivity of *An. stephensi* to different pyrethroids in the malarious regions of Iran, using pyrethroids is still suggested. Performing complementary tests with cyfluthrin on Bandar Abbas strain and with permethrin on Iranshahr strain seems to be necessary.

Key words: Anopheles stephensi, Iran, malaria, insecticide

INTRODUCTION

Malaria is one of the most important arthropod-transmitted diseases in Iran, so that more than 14000 cases of malaria have been reported in 2004.This disease is one of the main health problems of south and south east parts of Iran. Sistan and Baluchestan, Hormozgan and Kerman with Fars, are the provinces which are important in malaria transmission and imported cases. In these provinces, 5 main vector namely *An. stephensi*, *An. fluviatilis*, *An. superpictus*, *An .d'thali and An. culicifacies* are the main malaria vectors, and *An. stephensi* play the most important role and the chief vector of malaria.

There are different methods including biological-chemical and environmental management for combating malaria vectors. Chemical control had been the main method of combating against adult stage of vector in Iran since eradication era. This activity started since 1948 using DDT. Resistance of *An. stephensi* to DDT, dieldrin and malathion was reported in 1957, 1960 and 1976, respectively (Mofidi *et al*., 1958; Mofidi and Samimi, 1960; Manouchehri *et al*., 1976). Therefore, propoxur insecticide was replaced for Indoor residual spraying in 1978. Since 1992, lambdacyhalothrin an alpha-cyano pyrethroids was selected as the substitute insecticide and were used in some parts of the country. From the last year deltamethrin in the formulation of wettable powder was introduced.

Resistance of *An. stephensi*, especially its adult stage, against DDT, dieldrin and malathion have been widely reported in Persian-Gulf, Middle – East and Indian subcontinent regions (WHO, 1985, 1992). All relevant previous data on the status of *An. stephensi* to different insecticides in the country had been documented. In

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addition there are several studies in the resistant and susceptibility of this species to different insecticides around the world, where it play a role in malaria transmission, for instance, (Bansal and Singht, 2004) in India found the pyrethroids are more toxic to other insecticides to the larvae of this species; Hodjati *et al.* (2003) concluded that permethrin-resistant strain of *An. stephensi* exhibited less knock-down to pyrethroids than susceptible one. Results of Ganesh *et al.* (2003) revealed that resistant at the larval stage of *An. stephensi* is attributed to overproduction of esterases enzyme. Malcolm and Robinson (2001) reported high level (417-fold) of resistant to knock down and 3.3-fold resistant at mortality rate, when they were selected with DDT. In a parallel study was carried out by Mukhopadhyay *et al.* (1997), they showed that *An. stephensi* from Calcuta, India acquired resistant to DDT, BHC, propoxur and malthion, however the species is susceptible to deltamethrin. Kumar *et al.* (2004) selected *An. stephensi* at the larval stage with deltamethrin for 40 successive generations. They found high level of resistance (60-fold) at the larval stage and 6 fold at the adult stage. Spraying insecticides in human habitations severely restricts the number of suitable compounds which can be used in control programs. Using large quantities of insecticides, so that resistance can occur against them, is rapidly decreasing the number of suitable insecticides for malaria control. A better management strategy may be the use of compounds in rotational or mosaic alternation (Mellon and Georghiou, 1984). Using insecticides depends on largely the knowledge of the susceptibility levels of malaria vectors to currently used insecticides. This knowledge enables us to take all the necessary precautions to prevent the occurrence of resistance and to prepare a field plan for coping with it at the early stages of its development in advance. *An. stephensi* is the main malaria vector in malarious area of south and south-east parts of Iran. This study was carried out to determine the susceptibility levels of *An. stephensi* against different insecticides in three geographical regions including Hormozgan, Fars and Sistan and Babluchestan Provinces and test new insecticide, fipronil, belong to cyclodienes group.

MATERIALS AND METHODS

Study area: This study was conducted in three sites including: Iranshah, Kazeroon and Bandar Abbas during year 2004-2005 where insecticides have been intensively used and resistance against insecticides based on the National Malaria Control Program was supposed to be monitored (Fig. 1).

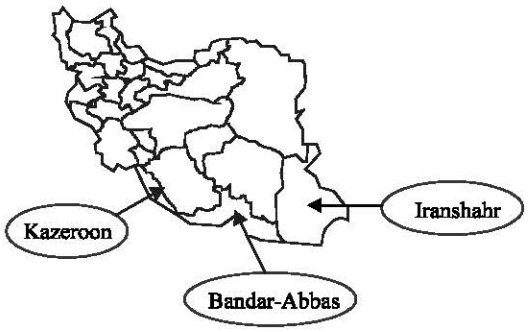

Fig. 1: Map of Iran and studied areas

Bandar abbas site: Bandar Abbas township, Hormozgan province, with surface area of 65,379 km^2 located between 25°, 24'-28°, 57' N latitude and 52°, 41'-59°' 15'E' longitude and the mean annual relative humidity of 50.5% and the maximum and minimum mean annual temperatures were 45 and 6°C, respectively.

Iran-Shahr site: Iran-Shahr Township, Sistan and Baluchistan province with surface area of 18750 km^2, located between 25°, 48'-63°, 19' N latitude and E longitude the mean annual relative humidity of 35% and the maximum and minimum mean annual temperatures of 51 and 0°C in July and December, respectively.

Kazeroon site: Kazeroon township, Fars province, with surface of 4119 km^2 located in east of Shiraz province between 51°, 35'-29°, 35'N latitude and E longitude and the maximum and minimum mean annual temperature of 15.5 and 30.1°C, respectively in September and October.

Mosquito rearing: The larvae and adults of 3 strains of *An. stephensi* were collected from malarious regions around of Bandar Abbas, Kazeroon and Iran-Shahr and transferred to the local insectary, where they were kept and fed with Bemax or fish food. Adults were kept in cages with different sizes. The females were allowed to feed weekly on guinea pigs or rabbit. Adult mosquitoes were fed with 10% glucose solution.

Adult bioassay: Bioassay was carried out on adult mosquitoes in insectarium at 27(±1)°C and 70 (±10)% relative humidity with a 12 h light and 12 h dark

period. Tests were carried out using WHO insecticide resistance test kits (WHO, 1988a). Six kinds of impregnated papers including , DDT 4%, dieldrin 0.4%, permethrin 0.75%, lambdacyhalothrin 0.05%, cyfluthrin 0.15% and deltamethrin 0.05%, supplied by WHO, but fipronil impregnated papers were prepared in the laboratory by spreading 2 mL insecticide (technical grade) in olive oil (as a solvent) by a pipette on Whatman No.1 filter paper (12×15 cm). All the impregnated papers were kept at refrigerator in a dark place before using. The technical grade of fipronil was supplied by Rhone-poulenc Agro.

Tests were carried out with 2-3 days' old unfed females. Batches of 22-25 females exposed to impregnated paper at least four times for different interval times. Tests with untreated papers were systematically run as controls.

The evaluations were performed in two steps. First, the required logarithmic times for killing between 5 and 99% of the adults in 24 h recovery period were determined. Secondly, 4-5 different times for yielding 5-99% mortality rates were evaluated for each insecticide; this is for plotting the baseline. Adult mortality rate was recorded 24 h after insecticide treatment. Results were corrected using Abbot's formula (Abbot, 1925) and log probit analysis of mortality rate was calculated by a computer based on Thomas and Sparks (1987). The 50% lethal time (LT_{50}) and the 99% lethal time (LT_{90}) were calculated for each insecticide. In some cases due to high resistant level, there was significant heterogeneity about the regression lines, but it was not possible to calculate confidence limits for the LT_{90} values.

RESULTS

The susceptibility tests were carried out with DDT, dieldrin, fipronil, permethrin, deltamethrin, lambdacyhalothrin and cyfluthrin in three geographical regions namely Bandar Abbas, Kazeron, Iran-Shahr. The results of susceptibility tests was based on decrease of mortality rates according to WHO criteria WHO (1988b), (98-100% mortality rates indicate susceptibility; 80-97% require verification and <80% indicate resistance). The response of susceptible Beech strain to 4% DDT, 0.4% dieldrin and 1% fipronil has been given in Table 1. The response of Beech strain to three insecticides in diagnostic dose was 98-100% mortality rate. This strain was obtained from London School of Tropical Medicine and Hygiene kindly provided by Professor C.F. Curtis. The LT_{50} value of this strain against DDT, dieldrin and fipronil, was 23.22, 20.56 and 19.13 min, respectively.

Table 1: Probit regression line parameters of susceptible reference strain of *An. stephensi.* (Beech) to DDT 4%, dieldrin 0.4% and fipronil 1% Bandar Abbas Iran (2004-2005)

			95% confidence interval		
			----------	----------	X^2 (df)
Insecticide	Intercept	Slope±SE	LT_{50}	LT_{90}	p-value
DDT	0.13	3.56±0.59			8.68(2)
			46.16	582.11	
			23.22	53.17	0.05
			12.26	31.72	
			40.28	542.11	
Dieldrin	0.39	3.5±0.59			8.86 (2)
			20.56	47.68	0.05
			10.30	28.38	
			22.19	82.61	
Fipronil	1.4	2.8±0.34			0.19 (2)
			19.13	54.82	0.05
			16.82	42.17	

The detail results of susceptibility test of *An. stephensi* against the seven insecticides used in three geographical sites are summarized as follows:

***An. stephensi*, bandar abbas strain:** In this geographical area, *An. stephensi* is resistant to DDT, dieldrin and fipronil and susceptible to deltamethrin, permethrin, lambdacyhalothrin and cyfluthrin insecticides. The mortality response of this strain against the discriminating dose of three insecticides namely DDT, dieldrin and fipronil was 20, 59 and 35%, respectively. The lambdacyhalothrin and cyfluthrin insecticides. Cyfluthrin had the lowest and deltamethrin had the highest mortality rates, based on LT50 value (Table 2).

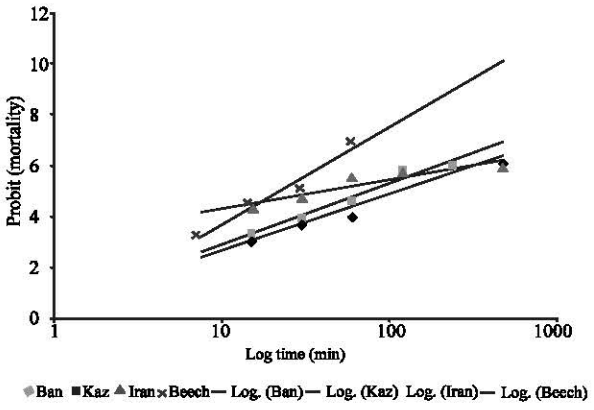

Fig. 2: Regression line of three strain of *An. stephensi* and susceptible Beech strain exposed to different interval times to DDT impregnated paper

Table 2: Probit regression line parameters of adult of *An. stephensi* (Bandar Abas strain) tested with different insecticides (2004-2005)

			95% confidence interval		
Insecticide	Intercept	Slope±SE	LT_{50}	LT_{90}	X^2 (df) p-value
DDT (4%)	0.05	2.20±0.36	183.13	1586.17	
			108.35	412.47	23.9 (4)
			66.81	229.48	0.05
Dieldrin (0.4%)	3.28	0.95±0.20	133.90	*	
			62.03	1352.00	15.57(4)
			21.89	401.12	0.05
Fipronil (1%)	1.58	1.69±0.26	200.82	*	
			102.30	580.47	8.168 (2)
			65.37	265.27	0.05
Deltamethrin (0.05%)	4.44	1.08±0.11	5.97	206.01	
			3.22	48.71	10.43 (4)
			1.80	20.91	0.05
Permethrin (0.75%)	4.05	1.64±0.24	8.42	123.92	
			3.75	22.70	22.33(4)
			1.91	9.76	0.05
Lambdacyhalothrin (0.05%)	4.16	1.38±0.14	7.35	115.69	
			4.04	34.11	12.17 (4)
			2.36	16.33	0.05
Cyfluthrin (0.15%)	4.07	1.24±0.07	6.97	91.77	
			5.46	58.03	6.90 (4)
			4.33	39.96	0.05

*LT_{90} is not shown, because it cannot be considered as reliable under this circumstance

Table 3: Probit regression line parameters of adult of *An. stephensi* (Kazeroon strain) tested with different insecticides (2004-2005)

			95% confidence interval		
Insecticide	Intercept	Slope ±SE	LT_{50}	LT_{90}	X^2 (df) p. value
DDT (4%)	0.38	2.46±0.31	110.050	660.00	
			74.590	247.30	8.55 (3)
			52.240	154.23	0.05
Dieldrin (0.4%)	2.95	1.26±0.27	84.610	*	
			41.100	420.15	11.07 (3)
			11.150	157.44	0.05
Fipronil (1%)	1.66	1.82±0.37	151.910	*	
			67.000	337.89	16.36 (3)
			32.580	149.93	0.05
Deltamethrin (0.05%)	4.73	1.18±0.8	2.110	31.93	
			1.670	13.89	6.32 (4)
			1.320	20.09	0.05
Permethrin (0.75%)	5.27	1.63±0.155	0.822	6.40	
			0.682	4.12	1.03 (4)
			0.562	3.00	0.05
Lambdacyhalothrin (0.05%)	3.69	1.57±0.21	14.570	205.22	
			6.800	44.46	19.83 (4)
			3.460	19.52	0.05
Cyfluthrin (0.15%)	4.11	1.25±0.108	6.935	84.75	
			5.062	52.89	7.77 (4)
			3.547	36.34	0.05(*)

*LT_{90} is not shown, because it cannot be considered as reliable under this circumstance

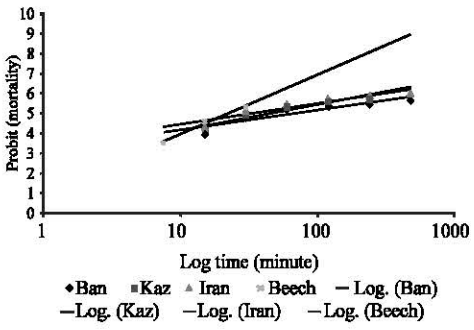

Fig. 3: Regression line of three strain *An. stephensi* and susceptible Beech strain exposed to different interval times to dieldrin, impregnated paper

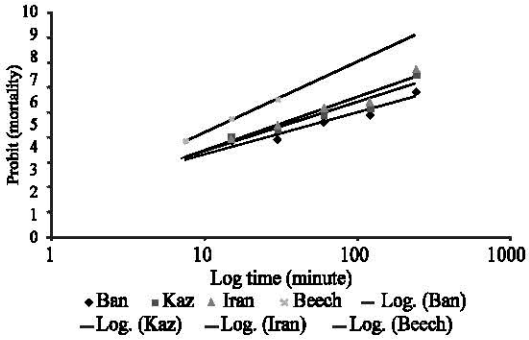

Fig. 4: Regression line of three strain of *An. stephensi* and susceptible Beech strain exposed to different interval time to fipronil impregnated paper

Table 4: Probit regression line parameters of adult of *An. stephensi* (Iranshahr strain) tested with different insecticides, (2004-2005)

Insecticide	Intercept	Slope ± SE	95% confidence interval LT_{50}	95% confidence interval LT_{90}	X^2 (df) p-value
DDT (4%)	0.69	2.53±0.34	73.75	429.14	
			50.01	160.44	10.17(3)
			32.89	101.44	0.05
Dieldrin (0.4%)	3.39	1.06±0.21	60.03	7575.00	
			31.83	508.00	14.19 (4)
			8.29	210.00	0.05
Fipronil (1%)	1.56	1.99±0.30	87.28	1044.95	
			54.14	238.41	10.25 (3)
			32.43	131.86	0.05
Deltamethrin (0.05%)	4.36	1.16±0.12	6.35	165.13	
			3.50	44.30	10.21 (4)
			2.06	20.02	0.05
Permethrin (0.75%)	4.00	1.40±0.08	6.38	62.30	
			5.10	41.88	8.85 (4)
			4.11	30.16	0.05
Lambdacyhalothrin (0.05%)	4.3	1.16±0.15	8.75	315.89	
			3.95	49.62	16.76 (4)
			1.93	18.87	0.05
Cyfluthrin (0.15%)	4.15	1.15±0.14	12.04	447.18	
			5.38	69.99	15.88 (4)
			2.68	26.22	0.05

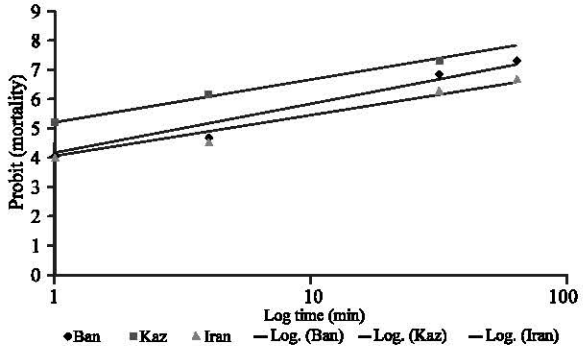

Fig. 5: Regression line of three strain of *An. stephensi* exposed to different interval times of permethrin impregnated paper

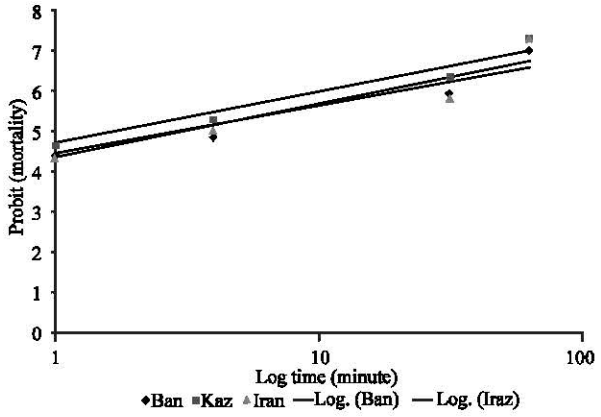

Fig. 6: Regression line of three strain of *An. stephensi* exposed to different interval times to deltamethrin impregnated paper

***An. stephensi*, Kazeroon strain:** The results of susceptibility test to DDT, dieldrin and fipronil insecticide with one hour exposure time showed that the population of this area is resistant to them and its mortality rate was 36, 65 and 45%, respectively. This strain was susceptible to the tested pyrethroids. The lowest and the highest mortality rates of this strain, based on LT_{50} value, were lambdacyhalothrin and permethrin (Table 3).

***An. stephensi*, Iran-Shahr strain:** This strain was resistant to DDT, dieldrin and fipronil insecticide with mortality rates of 63, 70 and 56%, minutes, respectively.

The LT_{50} value of the tested pyrethroids including deltamethrin, permethrin, lambdacyhalothrin and cyfluthrin was 3.50, 5.10, 3.95 and 5.38, respectively (Table 4).

Result of the performed tests on DDT, dieldrin and fipronil insecticide in the three geographical areas showed that the Bandar Abbas strain had the highest resistance level to them (Fig. 2-4).

Results showed that LT_{50} values of permethrin and deltamethrin have been 3.75, 0.68, 5.1 and 3.22, 1.67, 3.5 min for Bandar Abbas, Kazeroon and Iran-Shahr strains respectively, indicating significant different among them (Fig. 5 and 6).

The LT_{50} values of lambdacyholothrin and cyfluthrin against Bandar Abbas, Kazeroon and Iran-shahr strains are 4.04, 6.80, 3.95 and 5.46, 5.06, 5.38 at the LT_{50} against Bandar Abbas, Kazeron and Iran-Shahr, respectively (Fig. 7 and 8).

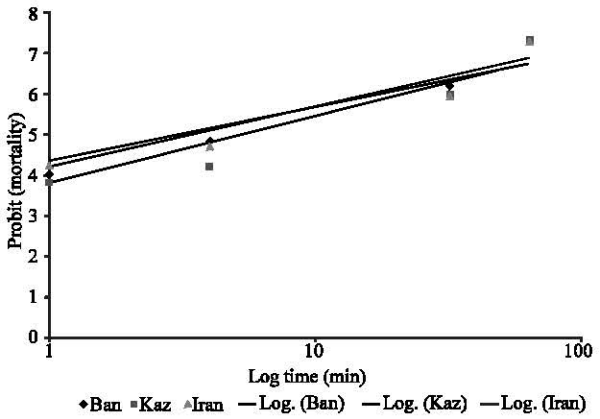

Fig. 7: Regression line of three strain of *An. stephensi* exposed to different interval times to lambdacyhalothrin impregnated paper

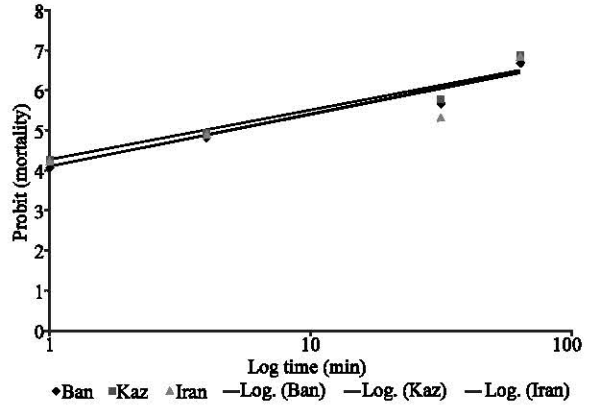

Fig . 8: Regression line of three strain of *An. stephensi* exposed to different interval times of cyfluthrin impregnated paper

DISCUSSION

An. stephensi with its long resistant history to different insecticides is an appropriate model studying resistance to insecticides in Iran. This species is considered sufficiently endophilic and endophagic to come into contact with insecticide residues in houses of urban regions. Therefore, careful and complete monitoring of malaria vectors for resistance against new insecticides should be a primary emphasis in malaria control activities.

Resistance is defined as the acquired ability of an insect population to tolerate doses of insecticide which kill the majority of individuals in a normal population of the same species.

The results of this study showed that *An. stephensi* is resistant to DDT 4%, dieldrin 0.4% and fipronil 1% in three geographical regions of Iran (Bandar Abbas, Kazeroon and Iran-Shahr); mortality rate from the diagnostic dose of DDT in those regions were 20, 36 and 66%, respectively.

These values were 59 65, 70% and 35, 45, 56%, for dieldrin and fipronil respectively. This resistance phenomenon in *An. stephensi* to DDT and dieldrin is attributed to the long history of using these organochlorine insecticides as indoor residual spraying for malaria control in main foci of disease since initiating of malaria eradication half a century ago.

Susceptibility of *An. stephensi* to DDT, had been tested in Iran in 1957 (Davidson and Jacson, 1961) Saudi Arabia in 1955 (Davidson, 1958), Iran in 1957 (Mofidi *et al*., 1958) and India (Mahan and Singh, 1965).

In Iran dieldrin resistance was first reported, in 1959, after 2 years starting its application (Zaim, 1987) . In 1974 it was reported that a field population of *An. stephensi* was resistant to DDT and dieldrin, but susceptible to malathion (Manouchehri *et al*., 1976) .

Adult *An. stephensi* was found to be susceptible to pyrethroids including permethrin, cyfluthrin, lambdacyhalothrin and deltamethrin in all regions. This susceptibility is confirmed, in spite of applying lambdacyhalothrin and deltamethrin from 1992 and 2003, respectively (Table 2-4). In Panaji, Goa, adults of *An. stephensi* were resistant to DDT 4.0%, dieldrin 0.4% and malathion 5.0% (Thavaselvam *et al*., 1993).

In Bikaner district, India, *An. stephensi* was found to be resistant to DDT and dieldrin and susceptible to fenitrothion and permethrin (Bansal and Singh ,1996). In Calculatta, west Bengal, *An. stephensi* was found to be susceptible to fenitrothion and resistant to DDT, dieldrin, malathion and propoxur (Chakraborty and Tandom, 2000). In Hormozgan province, southern of Iran, *An. stephensi* was found susceptible to bendiocarb, propoxure, malathion, fenitrothion, deltamethrin, permethrin, cyfluthrin and lambdacyhalothrin, but resistant to DDT (Vatandoost *et al*., 2005). A new study in adjacent to the Hormozghan province, known as Kerman province was undertaken. The results showed that this species is resistance to DDT and dieldrin but susceptible to other insecticides (Vatandoost *et al*., 2006).

In this study, it was appeared that resistance to DDT and dieldrin did not confer cross-resistance to pyrethroid. It was also determined in a study in Gujarat, west of India, that resistance of *An. culicifacies* to DDT and dieldrin, does not cause resistance to lambdacyhalothrin (Das *et al*., 1986).

Present results also showed the difference in slope of regression lines of resistant strains, than the slope of susceptible ones. Theoretically, a susceptible colony

which is composed of totally susceptible individual produces the highest slope for a regression line of dose-response data. With natural selective pressure from exposure to insecticides in the field, a population becomes heterozygous for resistant genotypes and as the frequency of resistant genotypes increases, the slope of the regression line drops off and the line will shift to the right (Theearaphap *et al*., 2002).

This study was the first large-scale survey that evaluated the susceptibility of different strains to fipronil. The results showed that resistance to dieldrin and fipronil were strongly associated in three strains of anopheline mosquitoes in Iran. It is mainly because of the identical gene of these mosquitoes. This observation is consistent with the results of a study of resistance to dieldrin and fipronil in two laboratory strains of *An. gambiae*, which indicated cross-resistance between these two insecticides (Brooke *et al*., 2000).

In one other study, two strain of *An. stephensi* including Beech and DUB_{234} were resistant to fipronil (Kolaczinski and Curtis, 2001). Such findings are very important particularly when the resistance mechanism confers resistance to more than one class of insecticides, as here with cyclodiene and phenyl pyrazole both acting on GABA receptor. Because of the cross-resistance relationship between dieldrin and fipronil in *An. stephensi*, in any field application of fipronil and dieldrin for controlling other anopheline mosquitoes, the possible pre-existence of polymorphism for dieldrin resistance should take into account. It is also likely that the use of fipronil increases the already high prevalence of the dieldrin resistance allele in the field population.

If fipronil is to be used for malaria control, the dieldrin resistance status of mosquito population needs to be examined first. More biochemical and molecular experiments are however required confirming the role of GABA receptor in resistance of mosquitoes to fipronil and dieldrin. This research is going to be continued to find this phenomenon.

In malaria endemic areas of Iran, performing comparative studies on susceptible and refractory populations for known malaria vectors are needed. This is especially true if there has been continuous intradomicillary spraying with pyrethroids and other insecticides. Additionally, such studies should be representative of other vectors of different geographical conditions and be conducted with greater frequency than in the past.

Meanwhile, use of pesticide in agriculture, should be in accordance with the issue of the resistance of malaria vectors and this activity should be monitored by Ministry of Health and Medical Education with collaboration of scientific centers.

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