Original Article

Irritability Levels of Field and Laboratory Population of *Culex pipiens* Complex in Tehran to Different Groups of Insecticides

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Abstract

Background: The irritant effect of some insecticides can cause a proportion of mosquitoes to leave the sprayed rooms before acquiring a lethal dose, so the repeated contact al sub-lethal dose may lead to extent the resistance.

Methods: Larvae and pupae of *Culex pipiens* complex were collected in mass from open canals of waste water in capital city Tehran and reared to obtain the first generation at laboratory. Sugar-fed 2–3 days female mosquitoes were used for the experiments and compared with laboratory strain. The irritability tests of insecticides impregnated papers were measured in plastic conical exposure chambers placed which implemented at controlled conditions according to the method described by WHO .Number of take-offs were counted during 15 minutes of exposure time.

Results: DDT had the most irritancy effect against field population of *Cx. pipiens*. DDT, permethrin and deltamethrin was moderately irritable against laboratory strain, whereas, addition to three previous insecticides, malathion, cyfluthrin and propoxur should be also considered as moderately irritable insecticides for field population of. Irritability level of etofenprox, fenithrothion, bendiocarb, and lambdacyhalothrin did not differ from control group.

Conclusion: The irritability response of mosquitoes may have a negative impact on control measures. Periodical execution of irritability tests with insecticides that routinely used in vector control program is highly recommended.

Keywords: Irritability, Insecticides, *Culex pipiens*, Tehran, Iran

Introduction

Culex pipiens complex has a great medical importance due to transmission of arbovirus and zoonotic diseases including *Dirofilaria immitis* (Azari et al. 2006). Also its biting and nuisance causes the severe allergies in humans and other hosts, and this led to discomfort especially in urban areas (Vinogradova 2000). The *Cx. pipiens* are known as a complex species including *Cx. pipiens*, and *Culex quinquefaciatus* are important members of *Cx. pipiens* complex (Azari et al. 2007, Harbach 2011). *Culex pipiens pipiens* are distributed in both temperate and tropical areas whereas *Cx. quinquefasciatus* in the tropical regions of the world (Harbach 1988, Smith and Fonseca 2004, Azari et al. 2010). *Culex pipiens* are spread in most parts of Iran, whiles *Cx. quinquefasciatus* are scattered in the southern of country (Zaim and Cranston 1986, Zaim 1987, Azari and Harbach 2009, Azari-Hamidian et al. 2010). Vectors control is facing many problems especially occurrence of insecticide resistance. Five major mechanisms have evolved to overcome toxi-

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cants such as reduces penetration, metabolic resistance, Target site insensitivity, excretion and behavioral change. In the behavioral change the mosquitoes are better able to resist the toxic effects of the insecticides, these changes, gradual resistance, gradually and inevitability increases the resistance genes in the population until all individuals are physiologically resistant (Thomson 1947, Vatandoost and Borhani 2004). The use of carbamate and phosphorus insecticides, especially in agricultural fields, was led to the development of this type of resistance to insecticides (Vatandoost et al. 2005a). Change of vector behaviors that occurs in different forms, so that it is possible to change the relaxing habits in the indoor and because of irritancy-repellency phenomenon due to some insecticides, which makes the insect does not rest long enough on spraying surface and thus, it does not receive a lethal dose. Therefore, it led to change the movement pattern of the mosquitoes into the houses and so they bitten out of the houses, avoids the insecticide-treated surfaces, occurrence of the exophagic and exophilic tendency and finally affected the results of vector control programs (Vatandoost and Borhani 2004). The mosquitoes could be avoided the spraying surfaces due to natural tendency and rest outside of buildings and houses (exophilic behavior). The irritation let to reduce to contact of mosquitoes with the sprayed surfaces, as well as absorption of enough dose of insecticides (Quinones and Suarez 1989). The different species of anopheline vectors have a different irritability level, some stay long time on insecticide-treated surfaces, while others motivated and escape immediately. Some vectors may be hyper-irritable e.g *Anopheles gambiae* that has 40–50 take-off during 15 minute's obligatory contact with DDT, whereas some other species may have hypo-irritable such as *An. albimanus* that has just 2–3 take-off in similar time, and some species are moderate-irritable, this phenomenon limits the residual spraying properties of the residential places against irritable species (Thomson 1947, Coluzzi 1963). The first studies on the irritability of *Anopheles* mosquitoes in Iran was conducted against *An. maculipennis* against DDT in Isfahan Province (Eshghy 1972). The other studies on the irritability of *An. stephensi* and *An. culicifacies* was determine against different insecticides was conducted in Sistan and Baluchistan Province (Vatandoost and Borhani 2004). It has been shown that resistant strains such as: *Anopheles gambiae* and *Cx. quinquefasciatus* demonstrate the different levels of irritability to the pyrethroids (Hougard and Duchon 2003). Reasonable and proper use of insecticides depends on the knowledge an awareness about sensitivity and irritability of the vectors to these insecticides. Many studies aimed to better understanding and effective application of the control methods of both larval and adult of *Cx. pipiens* complex (Oshaghi et al. 2007). Long-term use of insecticide may increase the resistance level of mosquitoes populations due to behavioral habits of mosquitoes and diversion from indoor to outdoor resting places (Thomson 1947). The irritability properties may change the mosquitoes behavior and led the leaving the indoor to outdoor which have been observed during the residual spraying of the villages with deltamethrin in endemic parts of malaria foci at southern east of Iran where the *An. culicifacies* and *An. stephensi* acts as main vectors (Abai et al. 1999, Alipour et al. 2005). Irritability is a first reaction of mosquito towards the sprayed environment, which initially increases movements of mosquitoes and finally led to away from the sprayed area, So repellency effect of insecticides is due to their irritability property (WHO 1970). The Cx. *pipiens* complex is main prevalent species at southern part of capital city of Tehran and easily bred in the high density at the sewage water as well as rice fields especially in the south parts where it reaches to this plateau (Vatandoost

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et al. 2005). Different insecticides are being used for household and agricultural pest control in the capital city of Iran, Tehran which may lead to enhanced tolerance of house mosquito to different Insecticides (Vatandoost et al. 2005). Due to rare studies on irritability of *Cx. pipiens* complex this study was designed to understand the irritability level of field strain of Capital City Tehran compared with laboratory TEH-SPH strain to different insecticides.

Materials and Methods

Different ages of larvae as well as pupae were collected using standard dippers from rice field and margin of open sewage canals where it reached to the plateau at the south part of the capital city of Tehran. The larval colony was transported to the insectary of School of Public Health (SPH), bred the immature using the habitat water and a few amount of flake fish food added daily to each enameled pan until adults emerged. Laboratory strain of *Cx. pipiens* (TEH-SPH) which was used as a reference in this study originally collected from capital city of Tehran and had been colonized in SPH insectary from 1960 for more than 50 years and never exposed to any insecticides.

Mosquitoes were maintained at 28–30 °C and 55–75 % relative humidity (RH). The light intensity in test-room was 5–8 foot-candles using two 40 W florescent lamps without any natural light and the reflected light reduced to a minimum. The tests were carried out between 9.0 AM and 5.0 PM. The emerged adults were fed with 5 % sucrose solution until 2–3 age mosquitoes which used for the tests.

The irritability tests were carried out according the instruction of World Health Organization (WHO) and the irritability test kit (WHO 1970). Due to exhibition of more take-offs of female mosquitoes than males (Rutledge et al. 1999), so only sugar-fed female mosquitoes were individually tested for the irritability level using both TEH-SPH strain and field population and exposed 15 min to WHO's impregnated papers inside the WHO's cone and the number of take-off was counted and mean and standard error was assessed as a criteria for irritability level. Due to direct correlation of mosquitoes take-off with temperature (Kaschef 1968), the tests were done at insectary condition with 29–30 °C and 55–75 % of RH and the light only provided with two 40 W florescent lamps and the intensity varied between 5 to 8 foot candle for laboratory environment without any natural light. The following insecticide impregnated papers of WHO were employed, DDT 4 %, bendiocarb 0.1 %, propoxur 0.1%, malathion 5 %, fenitrothion 1.0 %, permethrin 0.75 %, deltamethrin 0.05 %, lambdacyhalothrin 0.05 %, etofenprox 0.5 % and cyfluthrin 0.15 % and the oil impregnated papers were used for the control group. The mean of take-off was analyzed using ANOVA and independent t-test was separately for the treated and control groups according to field population and TEH-SPH strain under SPSS ver. 18.0.

Results

The irritability levels of were presented in Table 1,2 and Figs 1, 2,3 using TEH-SPH and field strains of *Cx. pipiens* complex according to different insecticides. The mean and standard error of take-off for DDT 4.0 % as an organochlorine pesticide was 11.2±1.5 and 2±0.2 respectively in both field and TEH-SPH strains. This insecticide should be regarded as a discriminative for the detection of irritability among mosquitoes population. From the organophosphate, fenitrothion 1.0 % and malathion 5.0 % were assessed and number of take-off per minute were 4.6±1.6 and 2.8±1.0 for the field strain and 1.3±0.7

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and 2.2±0.9 for TEH-SPH compared to 1.7± 0.3 for the control group. From carbamate, propoxur 0.1 % and bendiocarb 0.1 % were assessed for the irritability level against field and TEH-SPH strains of *Cx. pipiens* complex. The number of take-off per minute of propoxur 0.1 % and bendiocarb 0.1 % were 4.0±1.1 and 2.8±0.2 for the field strain and 3.6±0.2 and 2.9±0.2 compared to 1.3±0.7 for TEH-SPH strain. The irritability of both type I (permethrin 0.75%) and type II (deltamethrin 0.05 %, cyfluthrin 0.15% and lambdacyhalothrin 0.05%) as well as pseudo-pyrethroid (etofenprox 0.5%) were assessed. The irritability level for deltamethrin 0.05 %, cyfluthrin 0.15 %, lambdacyhalothrin 0.05 % were respectively 6.7±0.3, 4.1±0.2, 2.3±0.2 for the field strain and 3.5±1.4, 2.1±1.0 and 2.1±0.9 compared to 1.3±0.7 and 5.4±0.4 for TEH-SPH strain. The irritability caused by etofenprox 0.5 % as pseudo-pyrethroid was 2.0±0.2 and 1.5±0.8 for field and TEH-SPH strains. The number of take-off per minute for permethrin 0.75 % as pyrethroids type I was 6.1±0.3 and 4.1±1.5 for the field and TEH-SPH strains.

Table 1. Irritability levels of *Culex pipiens* (Tehran field strain) to different insecticides at laboratory conditions using WHO' kit and the impregnated papers

Insecticides	Number of Take off	Mean of take off in 15 minutes	Standard Error (SE)	Irritability level
DDT 4.0%	223	11.2	0.5	Moderately-irritable
Deltamethrin 0.05%	133	6.7	0.3	Moderately-irritable
Permethrin 0.75%	121	6.1	0.3	Moderately-irritable
Malathion 5.0%	95	4.8	0.3	Moderately-irritable
Cyfluthrin 0.15%	81	4.1	0.2	Moderately-irritable
Propoxur 0.1%	76	3.6	0.2	Moderately-irritable
Bendiocarb 0.1%	57	2.9	0.2	Hypo-irritable
Fenitrothion 1.0%	49	2.5	0.1	Hypo-irritable
Lambdacyhalothrin 0.05%	45	2.3	0.2	Hypo-irritable
Etofenprox 0.5%	39	2.0	0.2	Hypo-irritable
Control	538	1.9	0.3	Non-irritable

Table 2. Irritability levels of *Culex pipiens* (TEH-SPH strain) to different insecticides at laboratory conditions using WHO kit and impregnated papers

Insecticides	Number of Take off	Mean of take off in 15 minutes	Standard deviation (SE)	Irritability level
DDT 4.0%	132	4.4	0.9	Moderately-irritable
Permethrin 0.75%	123	4.1	0.9	Moderately-irritable
Deltamethrin 0.05%	106	3.5	0.8	Moderately-irritable
Malathion 5.0%	65	2.2	0.5	Hypo-irritable
Cyfluthrin 0.15%	63	2.1	0.5	Hypo-irritable
Lambdacyhalothrin 0.05%	64	2.1	0.5	Hypo-irritable
Etofenprox 0.5%	44	1.5	0.2	Non-irritable
Fenitrothion 1.0%	38	1.3	0.2	Non-irritable
Bendiocarb 0.1%	40	1.3	0.2	Non-irritable
Propoxur 0.1%	38	1.3	0.2	Non-irritable
Control	165	1.3	0.2	Non-irritable

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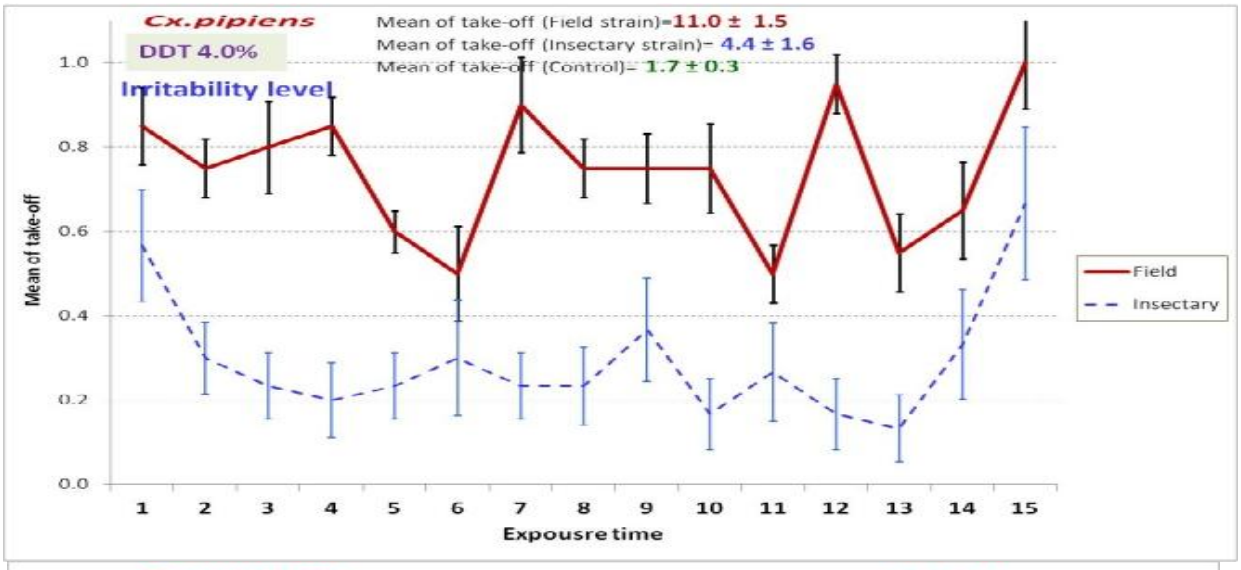

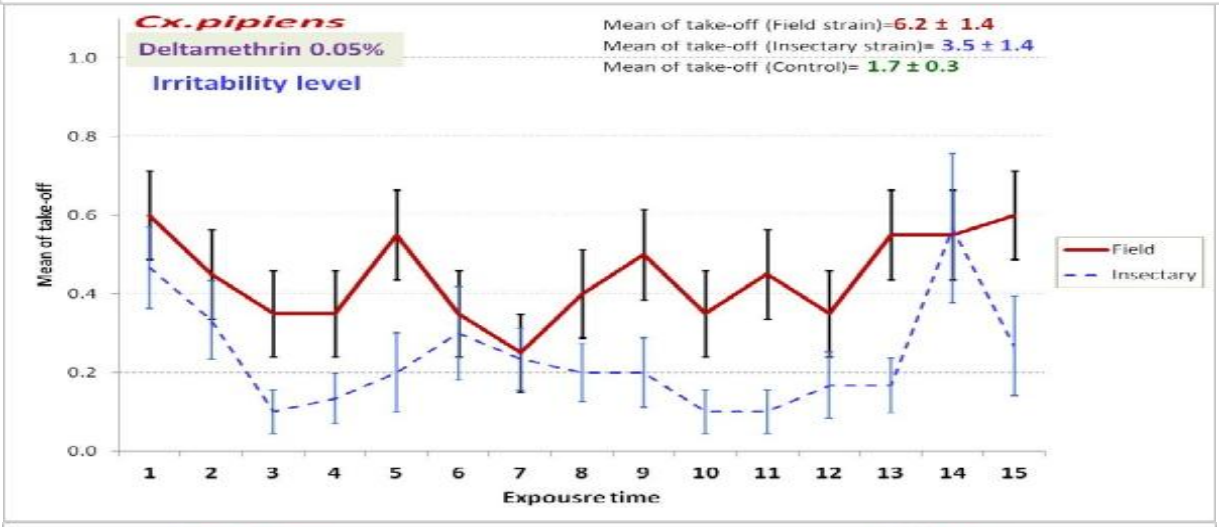

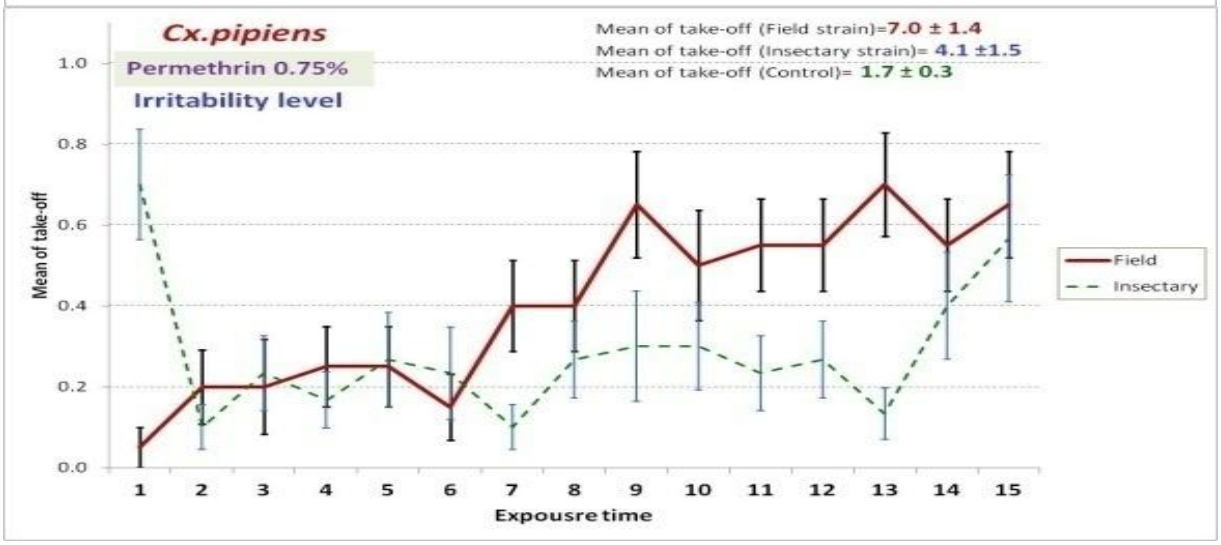

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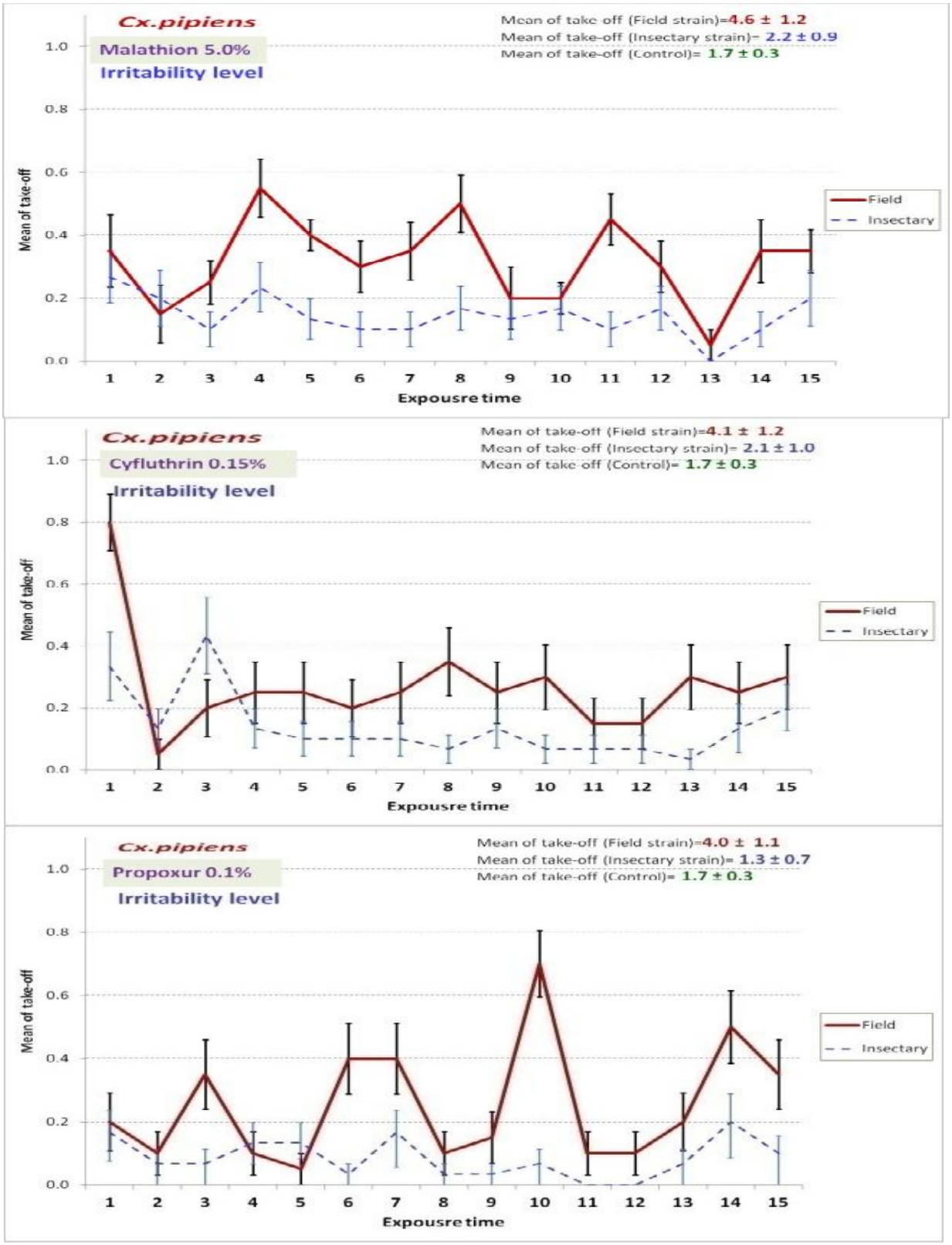

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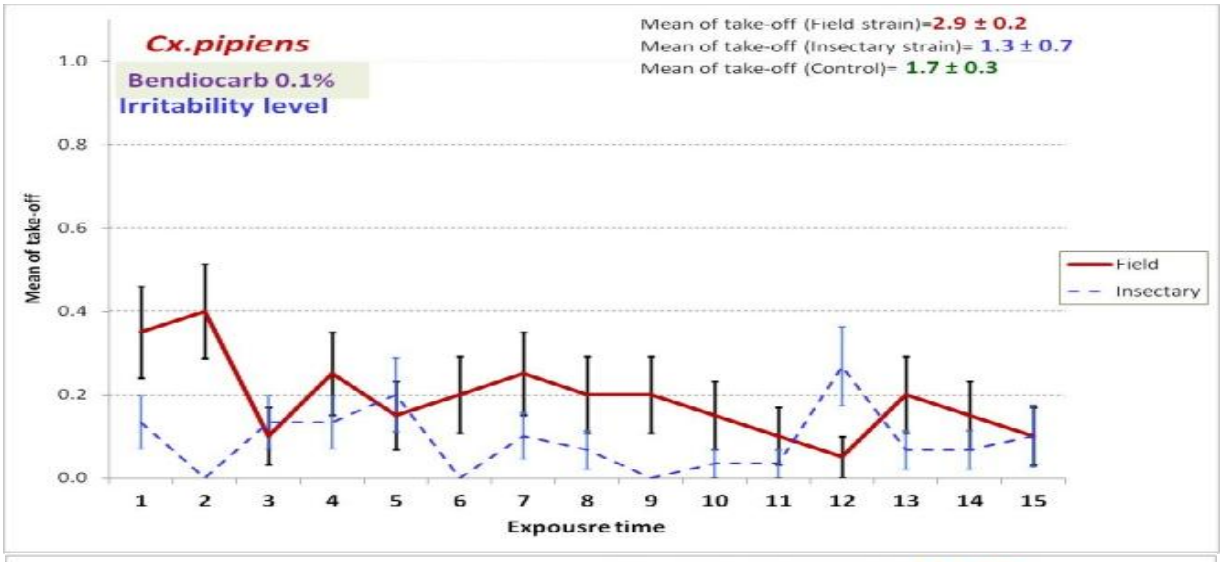

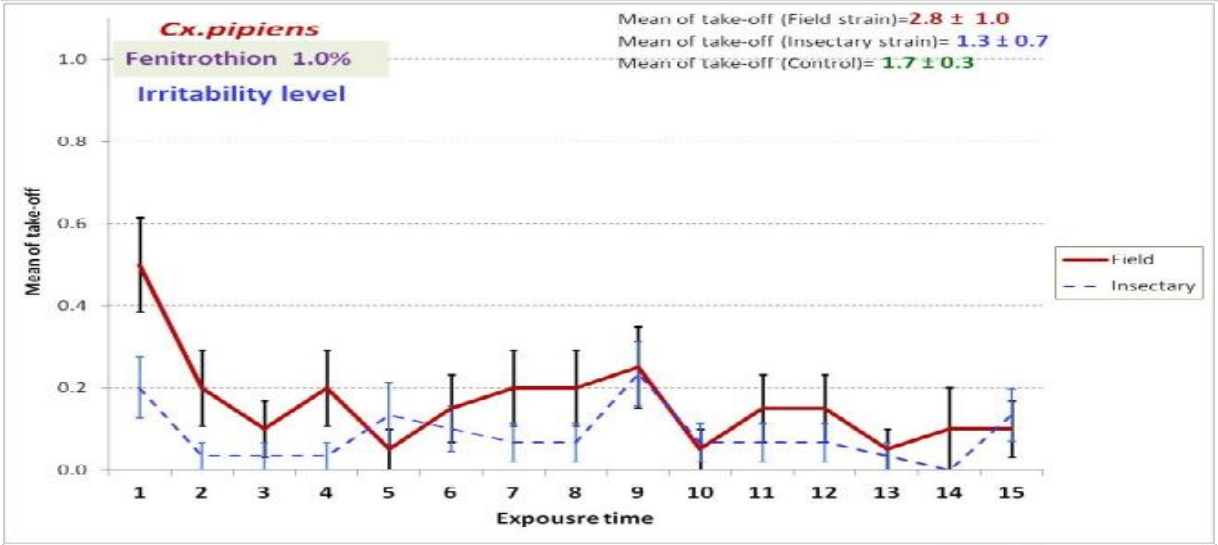

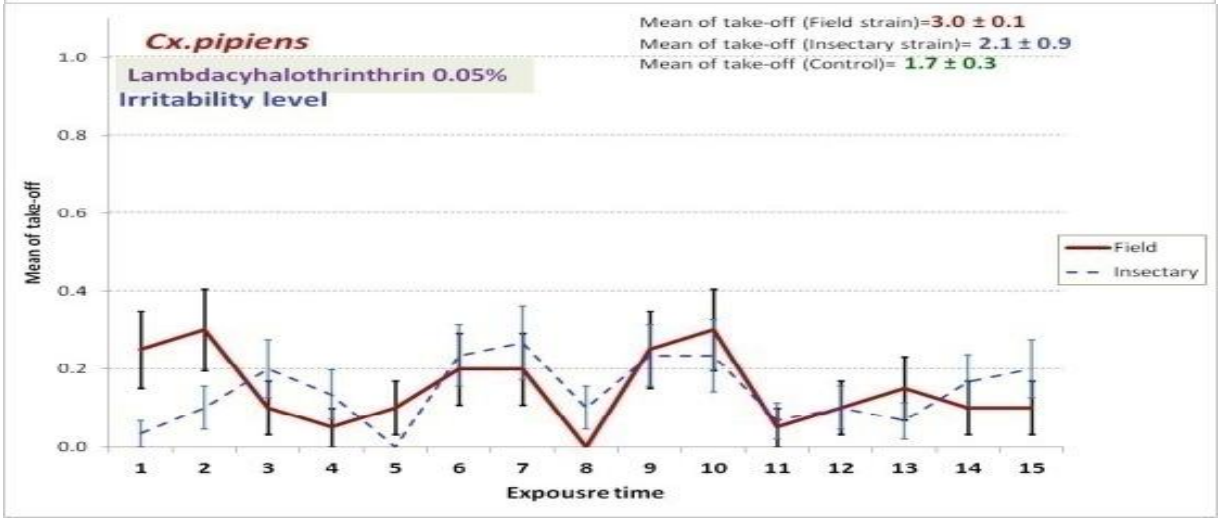

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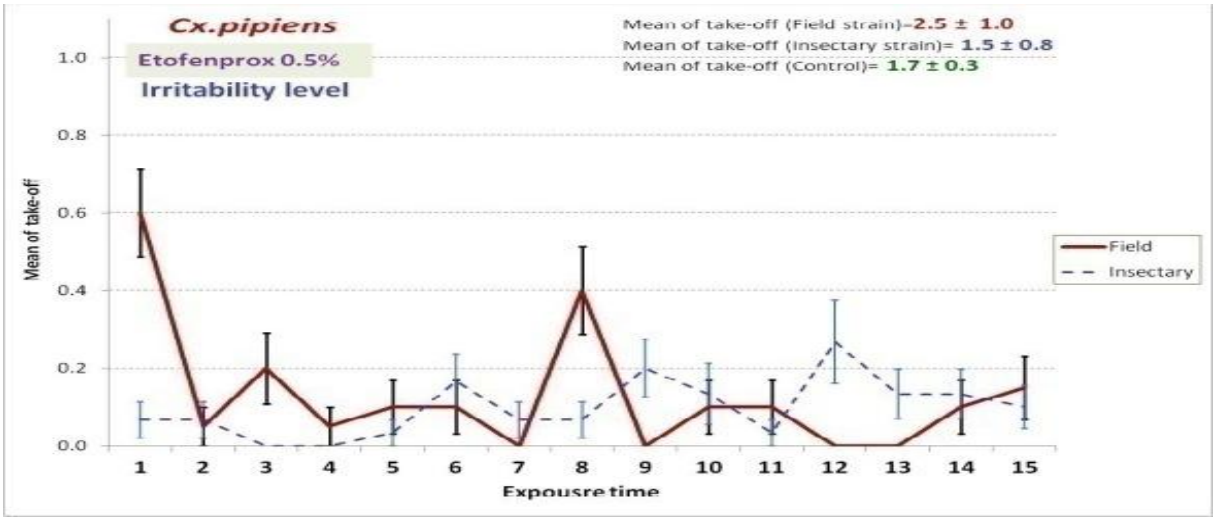

Fig. 1. Irritability levels of the field population compared with the TEH-SPH strain of *Culex pipiens* to 10 insecticides, Tehran, Iran

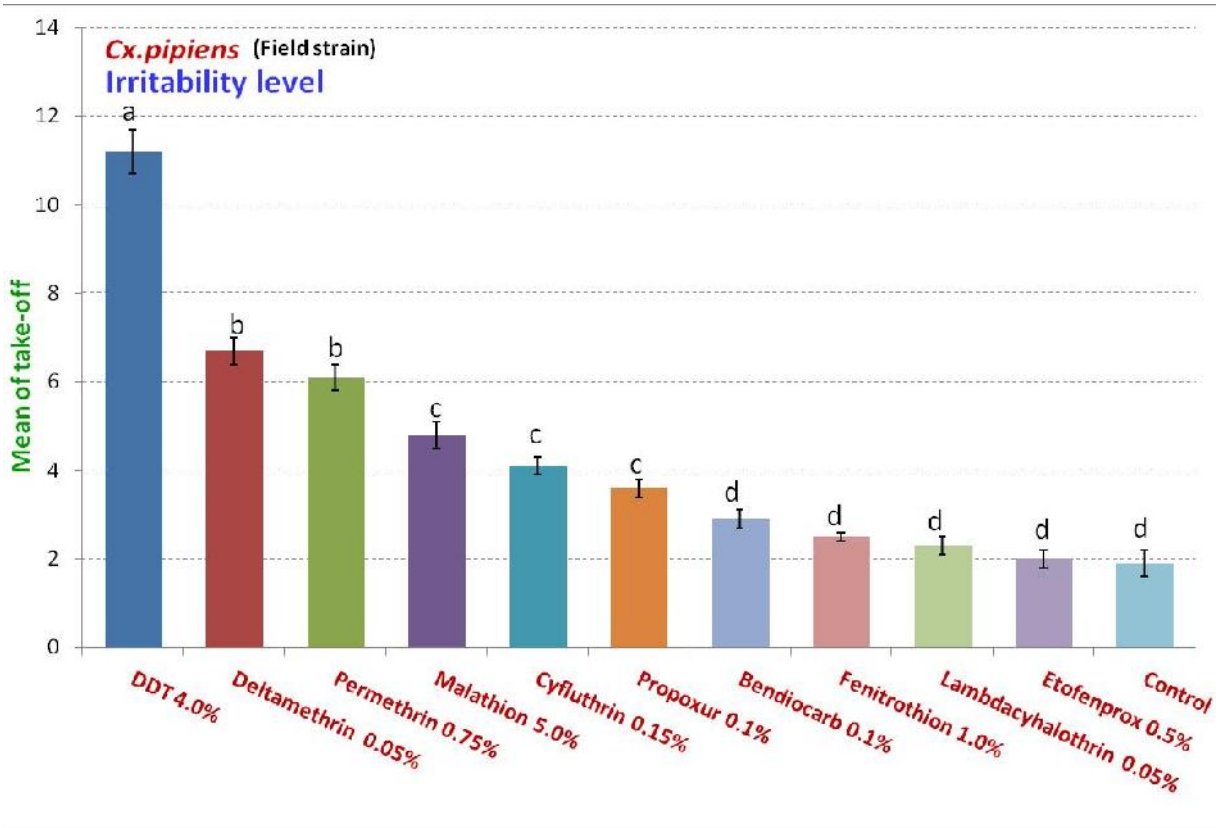

Fig. 2. Comparison of irritability mean of *Culex pipiens* (field strain) to different insecticides, Tehran, Iran

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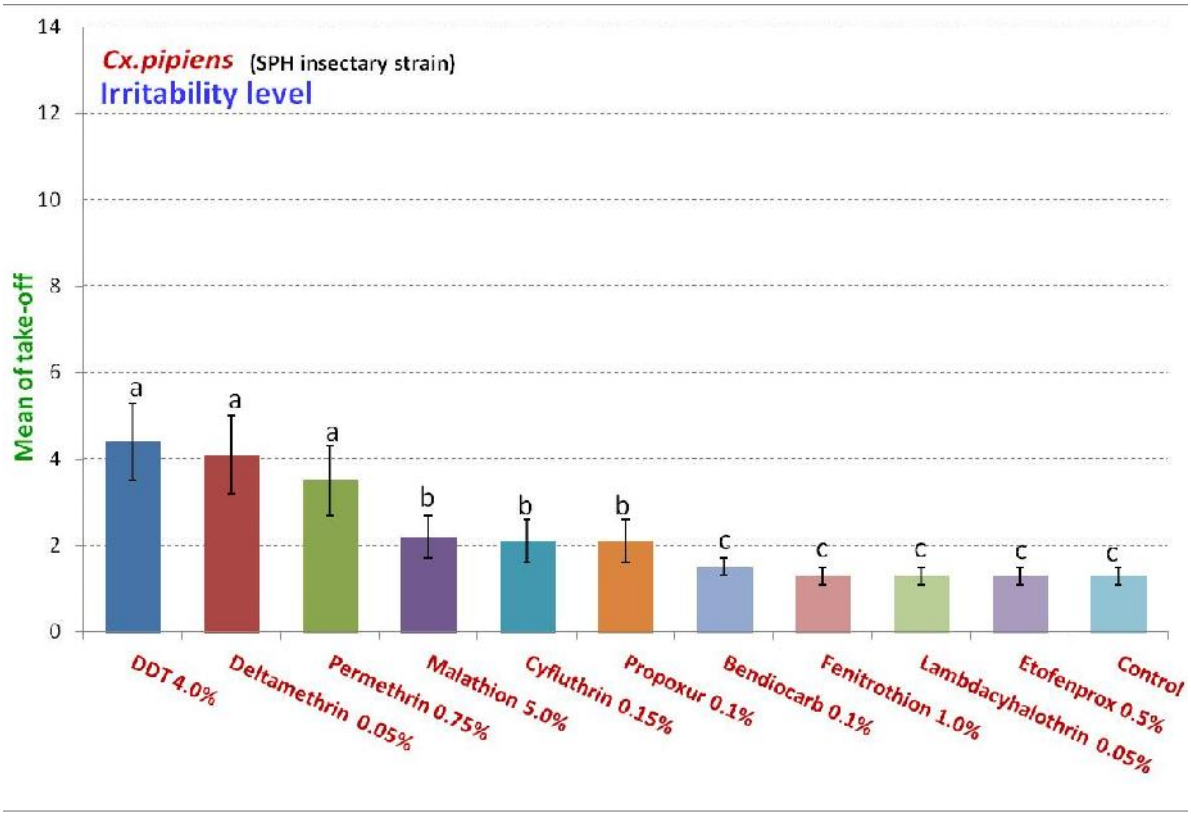

Fig. 3. Comparison of irritability means of *Culex pipiens* (TEH-SPH strain) to different insecticides, Tehran, Iran

Discussion

In Iran, many pesticides have been applied for the control of medically important arthropods in both the private and public health sectors. Various vector control measures including residual spraying (IRS), larviciding using *Bacillus thuringiensis* (BTi), impregnation of bed nets (ITNs) and long-lasting impregnated nets (LLITNs) have been employed in malaria foci. Currently the deltamethrin is one of the most commonly insecticide used in public health against malaria vectors in Iran since 1994. In recent years, the new evidence of pyrethroids resistance has been indicated with the increasing trend of tolerance among different species of the mosquitoes in Iran (Vatandoost and Hanafi 2012). The resistance history to insecticides has been notified during past years. Susceptibility tests which carried out during July-August 2000, the *Cx. pipiens* was highly resistant to DDT 4.0 % with LC_{50} value reported 6.8 % and the resistance range had been varied at both north and south parts of Tehran (Nazari and Janbakhsh 2000).

Expert committees of WHO has been classified the irritability level of mosquitoes to insecticides as hypo-irritable (2–3 take-off per minute), moderately irritable (3–30 take-off per minute) and hyper-irritable (30–40 take-off per minute) (WHO 1964). In this study, for the better statistical analysis and reduction of bias, the irritability tests were carried out at uniform conditions including fixed time (9 AM–5.0 PM), controlled tem-

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perature (29–30 °C), humidity (55–75%) and light intensity (5–8 fc) as well as the tested mosquitoes were sugar-fed, 2–3 ages and the first generation of the field population having definite strains of female *Cx. pipiens* were used. The locality of immature sampling was fixed in south part of Tehran during the mass collection.

A full series of irritability tests were carried out using 10 groups of insecticides including organochlorine, organophosphate, carbamate and pyrethroids against field population of *Cx. pipiens* in comparison with a THE-SPH strain. The finding concerning DDT-irritability tests revealed the moderately irritable level both for the field population (11.2±0.5) and the TEH-SPH strain (6.4±0.2). The take-off mean for DDT was recorded as the highest value in field strain and statistically differed from irritation caused by other insecticides ($P< 0.05$). Furthermore DDT, a series of irritability tests was carried out on the organophosphate (malathion 5.0%), one carbamate (propoxur 0.1%) and three pyrethroids (permethrin 0.75%, deltamethrin 0.05% and cyfluthrin 0.15%) which assessed moderately-irritable with the take-off means ranged between 3.5 % and 6.7 % against both insectary and field population of *Cx. pipiens*. The results of statistical analysis of irritability tests were shown in Fig. 1 and Fig. 2. DDT, permethrin and deltamethrin induced significantly more take-offs than other insecticides but mean of take-off for three latter insecticides did not differ from each other against both strains of *Cx. pipiens* (Fig. 2). Irritability tests for malathion 5.0 %, cyfluthrin 0.15 and propoxur 0.1 % also revealed the moderately irritable level. The irritability of remaining insecticides including bendiocarb 0.1 %, fenithrothion 1.0 %, lambdacyhalothrin 0.05 % and etofenprox 0.5 % did not significantly differed from control group and so assessed as non-irritable level.

DDT has two types of toxic effect on mosquitoes-an initial rapid knock down effect, followed by a lethal effect as well as an excito-repellent effect is shown (Hodjati et al. 2003). Irritability tests with the ″big-sized mosquito″ *An. maculipennis* showed that the mean of take-off for DDT 4.0 % was 26.4 and 19.4 for *An. superpictus* (Eshghi 1972). Under the large cage conditions DDT-resistant *An. stephensi* were more readily irritated by the insecticide than a susceptible strain of the same species (Eshghy 1977). Also the similar work with another big-sized mosquitoes *An. sacharovi* showed that DDT had the most and deltamethrin the least irritancy effect. The average number of take offs/fly/minutes were recorded 0.8±0.2, 0.7± 0.2, 0.5±0.2, 0.5±0.3, and 0.2±0.1, for DDT, permethrin, lambdacyhalothrin, cyfluthrin and deltamethrin respectively (Vatandoost and Abai 2012) All the above-mentioned three species showed variability in their irritability to different pyrethroids especially deltamethrin.

Results from DDT irritability assays also showed that organochlorine insecticides can induce behavior-modifying actions, such as contact irritancy and spatial repellency, which reduces man-vector contact, despite evidence of insecticide resistance within the test population (Achee et al. 2009). The irritability to DDT shown in some populations of *An. albimanus* and *An. nuneztovari* may reduce the effectiveness of residual applications of this insecticide by causing the mosquitoes to seek untreated surfaces and/or leave the house to rest outdoors and thus avoid a lethal dose (Quinones and Suarez 1989). It has been shown that *An. hyrcanus* had a low irritability to insecticides but high resistance to DDT and moderate resistance to propoxur, while the susceptibility to malathion and fenitrothion remained complete. It is supposed that the nature of adaptation to insecticide pressure is, to a great extent, determined by mosquito endophily or exophily (Sorokin

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and Mingaleva 1992). The behavioral avoidance to insecticides may play a significant role in reducing the selection pressure and thus occurrence and spread of insecticide resistance (Chareonviriyaphap et al. 2013).

The other irritability experiments which carried out in Kahnooj district, southeastern Iran, showed that DDT and permethrin had the most irritancy effect on *An. stephensi* and *An. dthali*. In contrast with the our results, DDT and deltamethrin showed the least irritancy effect against *An. stephensi* with 0.42+0.08 and 0.77+0.12 take-offs min/adult, respectively. However lambdacyhalothrin had the least irritancy effect against *An. dthali* with 0.096+0.02 take-offs/min/adult. The mean number of take-offs/min/adult with permethrin showed significant difference to DDT, lambdacyhalothrin, cyfluthrin and deltamethrin (Vatandoost et al. 2005b). Also in the similar area, Jiroft district, southeastern Iran, the low take-off for deltamethrin and the higher value for DDT were shown. The average number of take-offs per min per adult was 2.09± 0.13 for DDT, 0.581±0.05 for dieldrin, 1.85±0.08 for permethrin, 1.87± 0.21 for lambda-cyhalothrin, 1.53±0.13 for cyfluthrin, and 1.23±0.1 for deltamethrin (Abai et al. 2009). The irritability tests were carried out in a malaria–prone area, Iranshahr and Nikshahr districts which similar values were recorded for *An. stephensi* exposed to permethrin, deltamethrin, cyfluthrin and lambdacyhalothrin as 6.64±1.04, 3.11±0.67, 2.73±0.61 and 2.57±0.67, and *An. culicifacies* were 2.24±0.37, 1.44±0.38, 1.59±0.35 and 1.46±0.5, respectively (Vatandoost and Borhani 2004). The results of another investigation with *An. culicifacies* showed the most irritancy for DDT and lowest for permethrin and cyfluthrin in Iranshahr and Sarbaz district, southeastern Iran (Vatandoost et al. 2011). The other study with *An. stephensi* showed the most irritancy effect for lambdacyhalothrin, the moderate for cyfluthrin and the less irritancy for deltamethrin (Vatandoost 2001). Behavioral resistance appears more rapidly in endophilic species than exophilic ones (Muirhead 1960) and the *An. stephensi* has more endophilicity habit than *An. culicifacies* and so it is assumed that they are under insecticides pressure selection (Bhatia and Deobhankar 1963, Abai et al. 1999, Alipour et al. 2005). Irritability studies of resistant and susceptible strains indicated that the resistant strain is 2.8 times less irritable to permethrin in comparison with susceptible one (Vatandoost 2000) and the value of irritability may be differed with mosquito strains (Brown 1958).

In conclusion, based on the finding, conjoint implementation of irritability and susceptibility tests in problematic area recommended for assessment the possible changing of mosquitoes behavior such as diversion to untreated indoor shelters or resting at outdoors and in long term, reinforcement of exophilic population at the areas under insecticide pressure whether indoor residual spraying or impregnation of bed nets operations. The behavioral avoidance to insecticides may play a significant role in occurrence and spread of insecticide resistance.

Conclusion

Periodically experiments on irritancy of different insecticides against mosquito will provide a clue for Integrated Vector Management

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