Occurrence of high resistance to DDT in the field population of arboviruses vector *Culex pipiens* complex in Iran

Reza Zeidabadi Nezhad¹, Hassan Vatandoost¹,²*, Mohamad Reza Abai¹, Navid Dinparast Djadid³, Ali Raz³, Mohamd Mahdi Sedaghat¹, Ahmad Raeisi⁴

¹Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
²Department of Environmental Chemical Pollutants and Pesticides, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
³Malaria and Vector Research Group, Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran
⁴Malaria Control Unit, Center for Communicable Diseases Control, Ministry of Health and Medical Education, Tehran, Iran

ARTICLE INFO

**Objective:** To determine the susceptibility status of *Culex pipiens* complex to DDT.

**Methods:** Larvae of species were collected from different breeding places and then transferred to the insectary, reared at optimal condition and then F1 from the larvae were tested against DDT. Insecticide impregnated paper of DDT 4% was provided by the Ministry of Health and Medical Education of Iran, which was purchased from the World Health Organization (WHO). Susceptibility tests were carried out according the guideline of WHO. Female mosquitoes were exposed to different interval times of imagicide and then Probit mortality regression line was plotted. From that LT50 and LT90 values were calculated.

**Results:** The results of test indicated the higher resistance of *Culex pipiens* against insecticide. LT50 and LT90 values were determined as 78.39 and 305.24 h, respectively. According to the criteria of WHO, this species is resistant to DDT.

**Conclusions:** Accurately monitoring resistance status is essential to guide the rational use of insecticides. High resistant to DDT may be due to extensive use of pesticides for pest control in home and agriculture and may confer cross resistance to other insecticides. Further studies are required to determine the mechanisms of resistance using molecular and biochemical methods.

1. Introduction

The *Culex pipiens* (Cx. pipiens) complex are the primary vector of several diseases[1,2]. They are often resistance to insecticides[3]. Resistance to insecticides has been reported among a large number of vectors of diseases, including the *Cx. pipiens*[4]. DDT, an organochlorine, shares a distinctive steric profile with pyrethroid acidic moieties[5]and resistance to DDT often provides cross-resistance to pyrethroids[6]. Knockdown resistance (kdr) has been identified in many insect species of agricultural and public health importance[6,7]. The role of *Cx. pipiens* for transmission of *Dirofilaria immitis* (dog heart worm), West Nile and Sindbis viruses have been reported in Iran[8,9]. *Culex quinquefasciatus* from different parts of the world have been reported to be resistant to various insecticide classes[10-15]. In recent years, extensive studies have been carried out on different strains of *Anopheles stephensi*[16,17]. This study revealed a considerable increase in the resistance of *Cx. pipiens* to DDT in which previous studies indicated in lower rates[18]. Recent published paper indicates the importance of arboviruses vectors and their resistat status to different insecticides[19]. The aim of the present study was to determine the susceptibility status of this species to DDT.

2. Materials and methods

2.1. Study area

All mosquito samples were collected from various locations of Qarchak county, southern capital city of Tehran (Figure1). There are various open waste water canals as well as sewers and drainage...
ditches which act as main breeding places for Cx. p. complex.

![Map](image)

**Figure 1.** Map of study area.

2.2. Sample collection and species identification

The immature stages of *Cx. p. pipiens* were collected from different localities of the Qarchak county and then transferred to the insectary of School of Public Health (SPH), Tehran University of Medical Sciences.

2.3. Adult susceptibility test

The susceptibility tests were carried out on 2–3 days 10% sugar-fed female mosquitoes. The logarithmic exposure times (8–128 h) for estimation of 50 and 90 percent of the lethal times for the female mosquitoes were used. Mortality of exposed mosquitoes was recorded after 24 h.

2.4. Statistical analysis

Bioassay data were analyzed using Probit analysis program. The lethal time for 50% and 90% mortality (LT$_{50}$ and LT$_{90}$) values and their 95% confidence interval and Probit regression line parameters were determined.

3. Results

The data of susceptibility tests revealed that the field strains of *Cx. p. pipiens* complex did not show any mortality after 1 h exposure to diagnostic dose of DDT. The first mortality of mosquitoes (3.0%) was exhibited after 8 h exposure time. The mortality rate of 74.1% was occurred after 128 h exposure (equal to 5 days). The LT$_{50}$ and LT$_{90}$ values were 78.4 and 305.2 h. The regression line of mortality rate of female mosquitoes of Qarchakh strain of *Cx. p. pipiens* complex was calculated as $Y = -4.1119 + 2.1707X$ (Figure 2).

![Graph](image)

**Figure 2.** Probit regression line of females of *Cx. p. pipiens* exposed to DDT 4%.

4. Discussion

Insecticides have played an important role in control of disease vectors such as mosquitoes, sandflies, flea and lice. Resistances of important vectors against insecticides are constantly increasing worldwide[20]. Nowadays more than 100 species of mosquitoes have been shown resistance to at least one insecticide. More than 50 species are related to Culicinaceae[21]. Resistance to organophosphates and resistance to pyrethroid insecticides in *Culex* vectors were reported in the worldwide[22,23]. *Cx. p. pipiens* is an important public health pest that is responsible for the discomfort of people around the world, particularly in Africa and Asia, due to its ability to adapt to most existing habitat[24]. Based on the World Health Organization report, *Cx. p. pipiens* has been highly tolerant to organophosphate insecticides[25]. Surprisingly, *Cx. p. pipiens* are becoming resistant to insecticides more quickly than most other mosquitoes[26]. In this study, the present status of insecticide resistance of field strain of *Cx. p. pipiens* complex was assessed against DDT 4%. In order to assess the variation from past studies the present one based on published data. Based on previous studies *Cx. p. pipiens* complex in most of the world exhibited high resistance to insecticides[27,28]. During a study on susceptibility level of *Cx. p. pipiens* in southern and central parts of Tehran, it was shown that it may be resistant to DDT 4% with LC$_{50}$ = 6.8%. Resistance to pesticides due to mutations in the target location or due to changes in the detoxification occurs in the insect's body. In recent years, a new evidence of the emergence of resistance to pyrethroid with increased tolerance among different species of mosquitoes have been found in Iran. The results of susceptibility tests in five regions in Turkey showed that species is resistant to DDT from all regions. *Cx. p. pipiens* in the southeast of Iran were evaluated against DDT, propoxur, lambdacyhalothrin. They exhibited resistant to malathion, while sensitive and tolerant to deltamethrin. In a recent study in southern Tehran, fifty percent lethal time was recorded no mortality after 24 h exposure to DDT 4%[29,30]. In a study in recent year in Northwestern Iran, it was shown a mortality rate of 15.62% after 1 h exposure to DDT 4%[31]. Monitoring and mapping of insecticide resistance in this species is going on in different parts of country for decision making for its control[32,33].

The present study revealed an unexpected resistance to DDT among field population of *Cx. p. pipiens* of which their breeding places are waste water. It is suggested to conduct a molecular study to reveal the mechanisms of resistance as well as mutation in related genes.

Conflict of interest statement

We declare that we have no conflict of interest.
Acknowledgments

This project is financially supported by the Tehran University of Medical Sciences under code number 95-02-27-30344.

References

1] Nasci RS, Miller BR. Culicine mosquitoes and the agents they transmit. In: Beatty BJ, Marquardt WC, editors. The biology of disease vectors. Niwot: University Press of Colorado; 1996, p. 85-97.

2] Turell MJ. Members of the Culex pipiens complex as vectors of viruses. J Am Mosq Control Assoc 2012; 28(4): 123-6.

3] Cui F, Raymond M, Qiao CL. Insecticide resistance in vector mosquitoes in China. Pest Manag Sci 2006; 62(11): 1013-22.

4] Daaboub J, Cheikh RB, Lamari A, Jha IB, Feriani M, Boubaker C, et al. Resistance to pyrethroid insecticides in Culex pipiens pipiens (Diptera: Culicidae) from Tunisia. Acta Trop 2008; 107(1): 30-6.

5] O’Reilly AO, Khambay BP, Williamson MS, Field LM, Wallace BA, Davies TE. Modelling insecticide-binding sites in the voltage-gated sodium channel. Biochemical J 2006; 396(2): 255-63.

6] Hemingway J, Karunaratne SH. Mosquito carbamateylesterases: a review of the molecular biology and biochemistry of a major insecticide resistance mechanism. Med Vet Entomol 1998; 12(1): 1-12.

7] Soderlund DM, Knipple DC. The molecular biology of knockdown resistance to pyrethroid insecticides. Insect Biochem Mol Biol 2003; 33(6): 563-77.

8] Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E, Abai MR, Mobedi I, Linton LM, et al. Distribution and ecology of mosquitoes in a focus of dirofilariasis in northwestern Iran, with the first finding of filarial larvae in naturally infected local mosquitoes. Med Vet Entomol 2009; 23(2): 111-21.

9] Azari-Hamidian SH, Yaghoobi-Ershadi MR, Javadian E, Mobedi I, Abai MR. Review of dirofilariasis in Iran. J Guilan Univ Med Sci 2007; 15(60): 102-14.

10] Bisset J, Rodriguez M, Soca A, Pasteur N, Raymond M. Cross-resistance to pyrethroid and organophosphorus insecticides in the southern house mosquito (Diptera: Culicidae) from Cuba. J Med Entomol 1997; 34(2): 244-6.

11] Chandre F, Darriet F, Doannio JM, Riviere F, Pasteur N, Guillet P. Distribution of organophosphate and carbamate resistance in Culex pipiens quinquefasciatus (Diptera: Culicidae) in West Africa. J Med Entomol 1997; 34(6): 664-71.

12] Liu H, Cupp EW, Micher KM, Guo A, Liu N. Insecticide resistance and cross-resistance in Alabama and Florida strains of Culex quinquefasciatus. J Med Entomol 2004; 41(3): 408-13.

13] Sathananthop S, Paepon P, Supaphathom K. Detection of insecticides resistance status in Culex quinquefasciatus and Aedes aegypti to four major groups of insecticides. Troop Biomed 2006; 23(1): 97-101.

14] Kasai S, Shono T, Komagata O, Tsuda Y, Kobayashi M, Motoki M, et al. Insecticide resistance in potential vector mosquitoes for West Nile virus in Japan. J Med Entomol 2007; 44(5): 822-9.

15] Prideon JW, Pereira RM, Becnel JJ, Allan SA, Clark GG, Linthicum KJ. Susceptibility of Aedes aegypti, Culex quinquefasciatus Say, and Anopheles quadrimaculatus Say to 19 pesticides with different modes of action. J Med Entomol 2008; 45(1): 82-7.

16] Enayati AA, Vatandoost H, Ladonni H, Townson H, Hemingway J. Molecular evidence for a kdr-like pyrethroid resistance mechanism in the malaria vector mosquito Anopheles stephensi. Med Vet Entomol 2008; 17(2): 138-44.

17] Vatandoost H, Hanafi-Bojd AA. Indication of pyrethroid resistance in the main malaria vector, Anopheles stephensi from Iran. Asian Pacific J Trop Med 2012; 5(9): 722-6.

18] Lotfi MD, Manouchehr AV, Yazdanpanah H. Resistance of Culex pipiens pipiens to DDT in northern Iran, 1973. Bull Soc Pathol Exot 1975; 68(1): 91-9.

19] Corbel V, Achee N, Chandre F, Coulibaly MB, Dusfour I, Fonseca DM, et al. Tracking insecticide resistance in mosquito vectors of arboviruses: the Worldwide Insecticide resistance Network (WIN). PLOS Neglected Trop Dis 2016; 1: 1-4.

20] Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. Annu Rev Entomol 2000; 45: 371-91.

21] World Health Organization. Expert committee on filariasis, Fifth report. WHO technical report series, No. 821. Geneva: World Health Organization; 1992.

22] Chandre F, Darriet F, Darder M, Cuany A, Doannio J, Pasteur N, et al. Pyrethroid resistance in Culex quinquefasciatus from West Africa. Med Vet Entomol 1998; 12: 359-66.

23] Cheikh H, Ali-Haouas Z, Marqune M, Pasteur N. Resistance to organophosphorous and pyrethroid insecticides in Culex pipiens (Diptera: Culicidae) from Tunisia. J Med Entomol 1998; 35: 251-60.

24] Hougard JM, Duchon S, Darriet F, Zaim M, Rogier C, Guillet P. Comparative performances, under laboratory conditions, of seven pyrethroid insecticides used for impregnation of mosquito nets. Bull World Health Organ 2003; 81: 324-33.

25] World Health Organization. Expert Committee on Insecticides. Wld Hlth Org. techn. Rep. Ser., 265. Geneva: World Health Organization; 1965.

26] Hamon J, Mouchet J. [Insecticide resistance in Culex pipiens fatigans Wiedemann]. Bull World Health Organ 1967; 37: 277-86. French.

27] Mukhopadhyay AK, Sinha SN, Yadav RL, Narasimham MV. Susceptibility status of Culex quinquefasciatus in Patna to insecticides. Indian J Public Health 1993; 37(2): 57.

28] Corbel V, N’guessan R, Brégues C, Chandre F, Djogbenou L, Martin T, et al. Multiple insecticide resistance mechanisms in Anopheles gambiae and Culex quinquefasciatus from Benin, West Africa. Acta Trop 2007; 101(3): 207-16.

29] Salim-Abadi Y, Oshaghi MA, Enayati AA, Abai MR, Vatandoost H, Eshaghian MR, et al. High insecticides resistance in Culex pipiens (Diptera: Culicidae) from Tehran, capital of Iran. J Arthropod Borne Dis 2016; 10(4): 483-92.

30] Rahimi S, Vatandoost H, Abai MR, Raeisi A, Hanafi-Bojd AA, Rafi F. Irritability levels of field and laboratory population of Culex pipiens complex in Tehran to different groups of insecticides. J Arthropod Borne Dis 2016; 10(2): 178-83.

31] Naseri-Karimi N, Vatandoost H, Bagheri M, Chavshin AR. Susceptibility status of Culex pipiens against deltamethrin and DDT, Urmia County, West Azerbaijan Province, northwestern Iran. Asian Pacific J Trop Dis 2015; 5(Suppl 1): S77-9.

32] Fathian M, Vatandoost H, Moosa-Kazemi SH, Racisi A, Yaghoobi-Ershadi MR, Ali Oshaghi M, et al. Susceptibility of culicidae mosquitoes to some insecticides recommended by WHO in a malaria endemic area of southeastern Iran. J Arthropod Borne Dis 2014; 9(1): 22-34.

33] Ataie A, Moosa-Kazemi SH, Vatandoost H, Yaghoobi-Ershadi MR, Bakhshi H, Anjomruz M. Assessing the susceptibility status of mosquitoes (Diptera: Culicidae) in a dicrofilariasis focus, Northwestern Iran. J Arthropod Borne Dis 2015; 9(1): 7-21.