Original Article

Susceptibility of *Phlebotomus papatasi* (Diptera: Psychodidae) against DDT and Deltamethrin in an Endemic Focus of Zoonotic Cutaneous Leishmaniasis in Iran

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Abstract

Background: *Phlebotomus papatasi* (Diptera: Psychodidae) is the main vector of zoonotic cutaneous leishmaniasis (ZCL) in Iran. The nonstandard use of pesticides against pests, particularly in agriculture, indirectly has caused the development of resistance and, consequently, the threat of control measures in ZCL endemic areas. Up to 2023, several reports of resistance in *Ph. papatasi* have been declared in the Old World. The purpose of this study was to measure the lethal time (LT_{50} and LT_{90}) of *Ph. papatasi* sand flies in the ZCL endemic center of Esfahan to DDT and deltamethrin insecticides.

Methods: Sand flies were collected in Borkhar and were tested using WHO adult mosquito test kit against DDT 4% and deltamethrin 0.0002%. The sand fly's survival was recorded during exposure time in 225, 450|, 900, 1800, and 3600-seconds' intervals for DDT and Deltamethrin and they were allowed to recover for 24 hours. Then LT_{50} and LT_{90} were analyzed using probit software. *Phlebotomus papatasi* were identified using morphological keys and other sand flies' species were excluded from the analysis.

Results: The insecticide against female *Ph. papatasi* revealed hundred percent mortality when exposed to DDT 4% and deltamethrin 0.0002%. The LT_{50} and LT_{90} were 19.32 and 22.74 minutes for DDT 4% and 39.92 and 51.33 minutes for deltamethrin 0.0002% respectively.

Conclusion: Results of this study revealed that *Ph. papatasi* is still susceptible to DDT and deltamethrin. This data provides valuable knowledge to implement effective control strategies against ZCL main vector and help to manage insecticide resistance in the region.

Keywords: Insecticide; *Phlebotomus papatasi*; Susceptibility; Iran

Introduction

Rural or zoonotic cutaneous leishmaniasis (ZCL) has significant socio-economic negative effects on communities (1, 2). In the Old World, the reservoirs of the disease comprise various rodent species of the subfamily Gerbillinae (3–7), and the main vector of the disease is the *Phlebotomus papatasi* sand fly (Diptera: Psychodidae, Phlebotominae) (8). Leishmaniasis is present in the Old World's three continents of Asia (9–11), Africa (12) and Europe (13). The dispersion of sand flies is also affected by several socio-economic factors and environmental factors, including relative humidity, temperature, rainfall and land topography (14, 15). *Phlebotomus papatasi* is the main vector of cutaneous leishmaniasis in the Old World (6,

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16). In addition, in Iraq, this species has been introduced as a vector of visceral leishmaniasis as well (17). In Iran, *Ph. papatasi* is a domesticated species, is mostly caught in the plain, and is very fond of humid microhabitat like rodent borrow in the arid climate and heat, as well as blood-feeding on humans and rodents (18). The main seasonal activity of *Ph. papatasi* has been reported in August in Iran (9), in May in Saudi Arabia (19) and the United Arab Emirates (20), and in the months of August, October and November in North Africa (21). As the World Health Organization (WHO) reports, the control of vectors and reservoir hosts are recommended for the prevention of ZCL (22). Control of sand flies is performed by using insecticides and the control of reservoir hosts is done using rodenticides (22).

Control of vectors using insecticides has been applied in many countries mainly against mosquitoes and against other vector insects including sand flies indirectly (23). One of the reasons of the development of resistance in *Ph. papatasi* in countries is the result of non-standard and unprincipled use of insecticides (24). Management of insect resistance to insecticides is possible through resistance monitoring and insecticides evaluation. Bioassay tests recommended by WHO are the most practical and cheapest method to evaluate insecticide resistance (25). Bioassay tests in the form of WHO kits, and Control Disease Center (CDC) kits are used worldwide (26). The insecticide resistance in sand flies was first introduced by Kishore et al. (27), as well as Singh et al. (28). Studies until 2023 have announced several reports of resistance in *Ph. papatasi* in the Old World (24, 29), but no community study has been done in Borkhar County, Esfahan Province, Iran. The aim of this study is to investigate the susceptibility of *Ph. papatasi* in this endemic center of Esfahan Province.

Materials and Methods

Study area

Phlebotomus papatasi were collected from Borkhar County of Esfahan Province in summer 2022 (Fig. 1). This county is a known ZCL focus and historically, indoor residual spraying (IRS) has been used in the area to control the malaria disease (30). Also, various pesticides are being used to control agricultural pests in the region. Borkhar County is located at 32°48'58.7" North 51°56'21.1" East. The height of this city is 1595 m above sea level and its area is 2180 km^2.

Susceptibility test

During the night, sand flies were collected from inside dwellings (for example, house, toilet, poultry farm, stable), and surface outsides of parked cars using a mouth aspirator and transferred to a cage that was previously placed in a plastic bag to supply humidity and temperature. In the laboratory, after two hours resting, the sand fly specimens were prepared for the sensitivity tests. The tests were performed with the papers impregnated with deltamethrin 0.0002 % and DDT 4% with the standard method of the WHO (25). Control tests were also performed based on the standard method of evaluating pesticides of the WHO Pesticide Evaluation Scheme (WHOPES) and using paper impregnated with acetone and silicone oil (0.66 ml of oil + 1.34 ml of acetone). In order to perform the susceptibility test in each test tube, 25 female sand flies (non-blood fed) were exposed to the test papers for 225, 450, 900, 1800 and 3600 seconds. The experiments were performed according to the guidelines of the WHO (26) and in four replicates for the treatment groups and the control group (Fig. 2). After the exposure times, the tubes were placed in the box with standard insectarium conditions (temperature 28±5 °C and relative humidity 70±5%) and after 24 hours the mortality rates were recorded. Then the tubes with live sand flies were transferred to the freezer for five minutes. Two groups of live and dead sand flies have been identified separately using morphological characteristics (31, 32), and *Ph. papatasi* species were separated and used for further analysis.

Data analysis

After the test, the mortality rates were modified using Abbott's formula. LT_{50} and LT_{90} were plotted using Finney 1971 probit software. Excel software was also used to draw Tables and Figures.

Results

In this study, 1470 females *Ph. papatasi* were tested. Of these, 973 *Ph. papatasi* were exposed to insecticides and 497 specimens were used as control group. The mortality rate of the sand fly specimens against DDT 4% and deltamethrin 0.0002% in five different times is shown in Table 1. The linear regression line and probit of *Ph. papatasi* mortality rate against deltamethrin 0.0002% and DDT 4% singularly and comparatively were drawn in the figures 3–5, respectively. LT_{50} and LT_{90} were 19.32 and 22.74 minutes for DDT 4% and 39.92 and 51.33 minutes for deltamethrin 0.0002%, respectively.

Table 1. The results of the sensitivity test of *Phlebotomus papatasi* against DDT 4% and deltamethrin 0.0002%, Borkhar County, Esfahan Province, 2022

n	Pesticide	Time (S)	No. tested	Live after 24 hours		Dead after 24 hours		Abbot
				Number	%	Number	%	
	Deltamethrin	3600	100	0	0.00	100	100.00	100
1	**DDT**		99	0	0.00	99	100.00	100
	Control		98	92	93.90	6	6.10	-
	Deltamethrin	1800	96	1	1.10	95	98.90	98.9
2	**DDT**		100	4	4.00	96	96.00	95.7
	Control		95	88	92.60	7	7.40	-
	Deltamethrin	900	95	19	20.00	76	80.00	77.8
3	**DDT**		99	9	9.10	90	90.90	89.9
	Control		103	93	90.30	10	9.70	-
	Deltamethrin	450	95	33	34.70	62	65.30	62.3
4	**DDT**		97	39	40.20	58	59.80	56.3
	Control		101	93	92.10	8	7.90	-
	Deltamethrin	225	97	59	60.80	38	39.20	31.6
5	**DDT**		95	59	62.10	36	37.90	30.2
	Control		100	89	89.00	11	11.00	-

Fig. 1. Geographical location of Borkhar County, Esfahan Province, Iran (Prepared by the first author)

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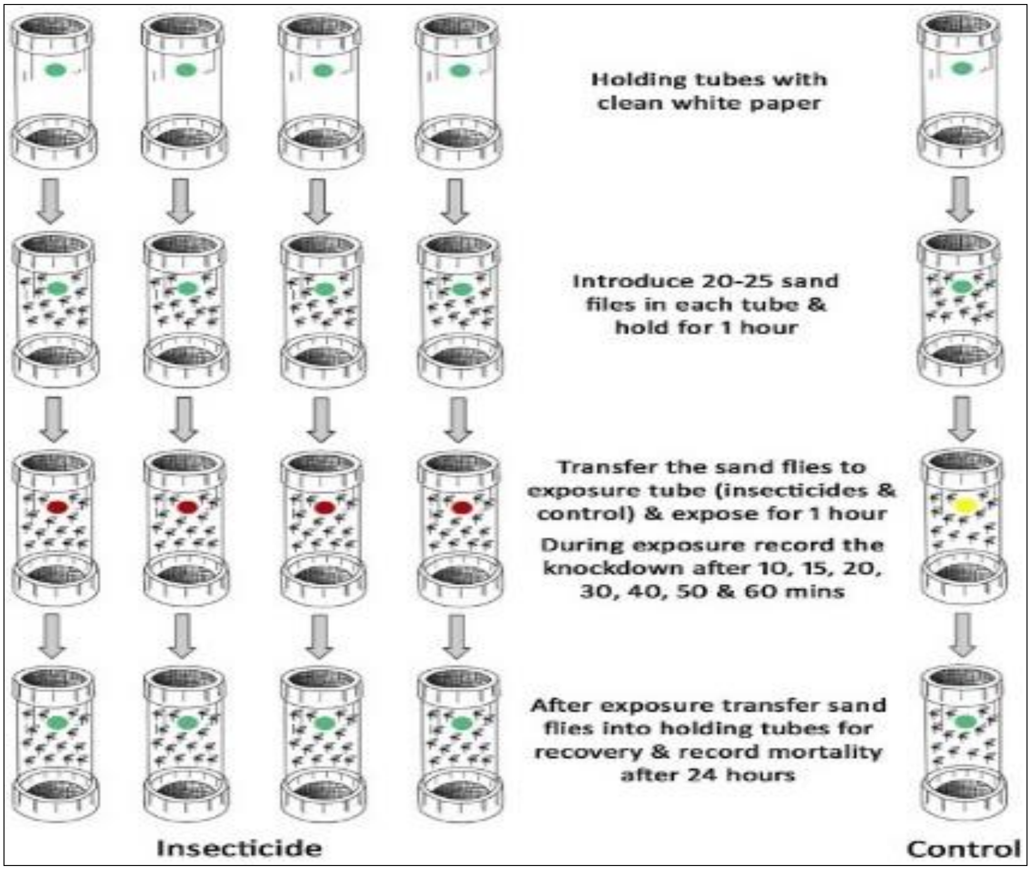

Fig. 2. A schematic view of WHO pesticide bioassay test which were used for susceptibility of adult *Phlebotomus papatasi* from Borkhar County, Esfahan Province, 2022 [Figure adapted from WHO (26)]

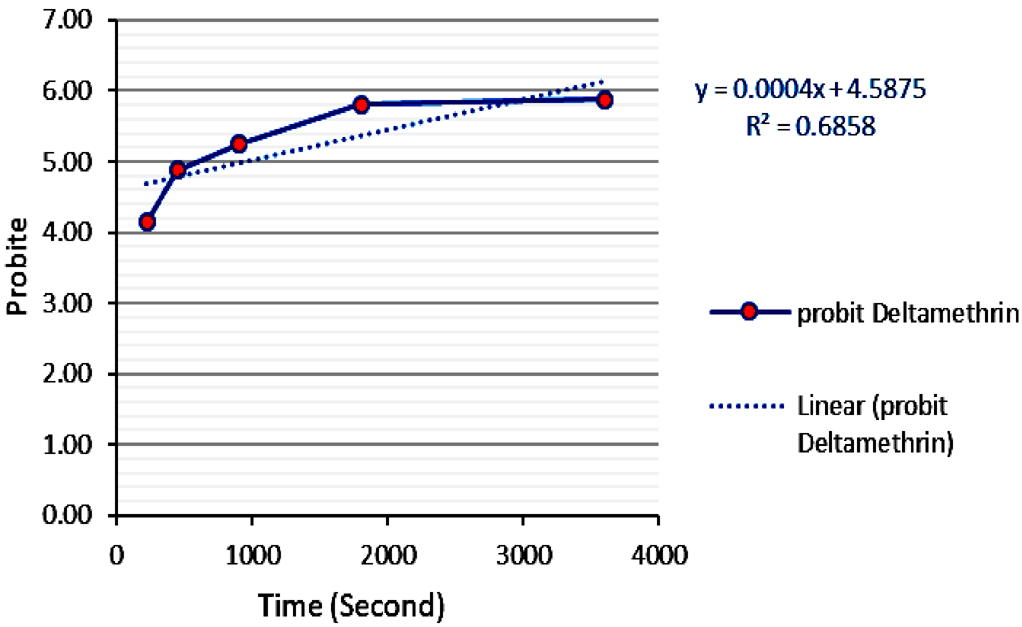

Fig. 3. Probit regression line of deltamethrin 0.0002% against *Phlebotomus papatasi* females, Borkhar County, Esfahan Province, 2022

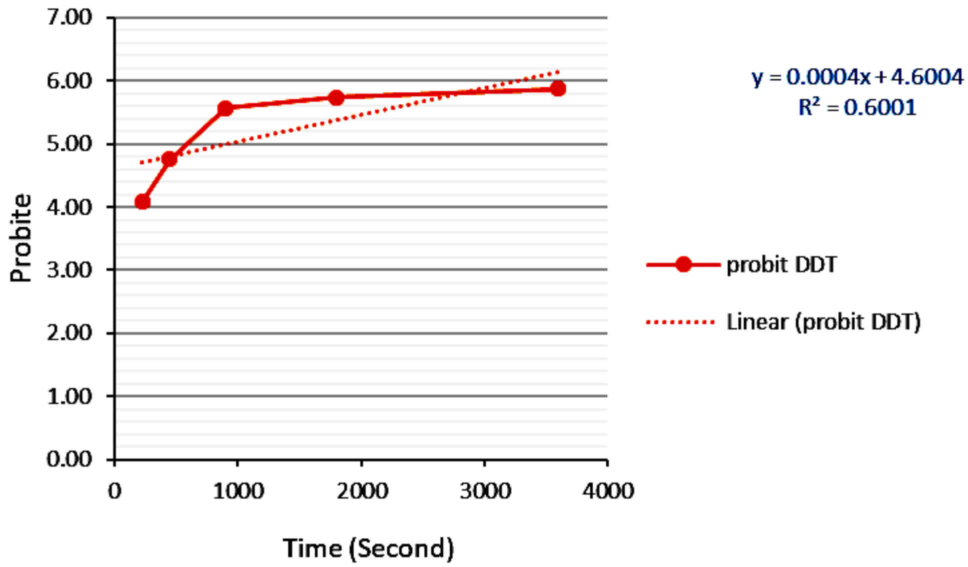

Fig. 4. Probit regression line of DDT 4% against *Phlebotomus papatasi* females, Borkhar County, Esfahan Province, 2022

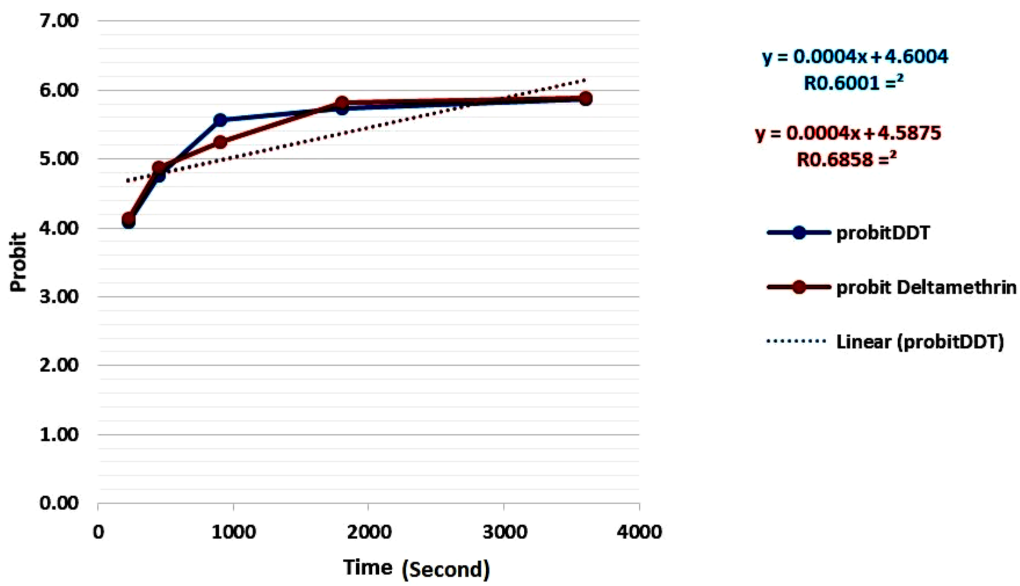

Fig. 5. Probit regression lines of DDT 4% and deltamethrin 0.0002% against *Phlebotomus papatasi* females, Borkhar County, Esfahan Province, 2022

Discussion

Sand flies have small size, discontinuous flight behaviors, growth and reproduction habitats, and blood-feeding behavior, which makes them part of the first group of insects exposed to insecticides (33). The use of insecticides in leishmaniasis control programs is inevitable (34). Controlling insect pests in agriculture, indirectly reduces insecticide susceptibility of medically important vectors of diseases, consequently, increase disease cases, and also has serious side effects such as environmental pollution (35). Infectious diseases are a major public health problem, especially in tropical regions in the world. There are several reports of sand fly resistance to chlorine insecticides, especially DDT 4%, and with a smaller percentage of malathion and pyrethroids in the world (35–37). Effective and successful control of leishmaniasis programs is of high value, subject to the evaluation of the sensitivity and resistance of sand flies to insecticides in order to design an effective control program (38). This study successfully evaluated the sensitivity/resistance status of sand flies of Borkhar City, Esfahan Province, and showed that sand flies of *Ph. papatasi* are still susceptible to DDT 4% and deltamethrin 0.0002%.

In this study, deltamethrin 0.0002% and DDT 4% insecticide concentration papers were used along with WHO insecticide holding tubes to check the sensitivity of *Ph. papatasi*. In a similar study in 2012 in Morocco, *Ph. papatasi* and *Ph. sergenti* were sensitive to DDT 4% and lambda-cyhalothrin 0.05% (39). The results of this study on DDT 4% were the same as our results. Similar study in Sudan showed that in some areas of this country, *Ph. papatasi* is sensitive to DDT 4%, permethrin 0.75%, malathion and propoxur insecticides, but in some other areas of the country, it was become resistant to malathion and propoxur (22, 40). In that study, it was suggested that the *Ph. papatasi* resistance to these two insecticides was due to the use of different pesticides for malaria control programs (40). In a study in two different area of Turkey, sand flies were resistant and sensitive to deltamethrin 0.05% and permethrin 0.75%, respectively (41). The resistance in the sand flies in that study was due to the long-term use of both insecticides in the region. The results of various studies have shown that sand flies have different levels of resistance to pesticides (37, 41). In a study in 1992, Sayedi-Rashti et al. (42) showed that *Ph. papatasi* in Esfahan Province is more tolerant to DDT 4% than other regions of the country and will probably become resistant to DDT 4% sooner than other insecticides. In the studies of Yaghoobi-Ershadi et al. (43) the results showed that until 2006, *Ph. papatasi*, in Borkhar County, was sensitive to deltamethrin 0.05% and in Badroud area to DDT 4%, also showed that the mortality rate of *Ph. papatasi* against DDT 4% was 88.8%. In Orzoyeh, Kerman Province, *Ph. papatasi* was sensitive to DDT 4% (44). Rashti et al. (42) also showed that *Ph. papatasi* was sensitive to DDT 4% in most rural cutaneous leishmaniasis foci in Fars Province. In a study in 2011 in Dehbakari City of Kerman Province, *Ph. papatasi* was 100% sensitive to DDT 4% and deltamethrin 0.05% (45). The results of a similar study in 2020 in Lorestan Province showed that this species was resistant to DDT 4% but sensitive to bendiocarb 0.1%, permethrin 0.75%, deltamethrin 0.05% and cyfluthrin 0.15% (35).

Among important sand fly vectors of leishmaniasis, *Phlebotomus argentipes* resistance to DDT 4% was reported for the first time in the Old World and in Bihar Province of India (46). In a study conducted between 2010 and 2016 in endemic areas of visceral leishmaniasis in India, *Ph. argentipes* was resistant to DDT 4 % and sensitive to deltamethrin 0.05% and malathion 5% (47). The results of this study on DDT 4% were not the same as the results of our work.

It is worth mentioning that *Ph. papatasi* sand flies of Isfahan Province are infected to *Wolbachia* bacteria (48), where this bacterium is involved in *Ph. papatasi* susceptibility to deltamethrin insecticide (49). If resistance to the currently used insecticides develops in the future, alternative vector control methods such as microbial approaches should be evaluated (50–52).

Conclusion

Numerous reports of the resistance of sand flies to pesticides have been proposed as a threat to the discussion of reduction and control of Leishmaniasis. Having sufficient information on the level of resistance of sandflies, continuous monitoring, and having a map of resistance

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to insecticides in Iran can be a warning for the health system and a good guide for vector disease control. The bioassay method using World Health Organization or CDC kits is one of the best methods for evaluating the resistance of insects to insecticides. Knowing the level of sensitivity of sand fly to different classes of pesticides makes it possible to implement effective control strategies at the right time and also prevent the spread of resistance to insecticides to some extent.

In order to maintain the epidemiological effectiveness of vector control interventions, regular monitoring of insecticide resistance in wild populations of vector sand flies is necessary.

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Ethical Consideration

The protocols were conducted in this study followed the guidelines of the institutional ethical committee (Tehran University of Medical Sciences, TUMS). The protocols were approved by TUMS ethical committee under registry IR. TUMS.SPH.REC.1400.267.

Conflict of interest statement

The authors declare there is no conflict of interests.

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