Original Article

Evaluation of Susceptibility Status of *Phlebotomus papatasi*, the Main Vector of Zoonotic Cutaneous Leishmaniasis, to Different WHO Recommended Insecticides in an Endemic Focus, Central Iran

Zahra Saeidi[1], Hassan Vatandoost[1,2], Morteza Zaim[1], Reza Ahmadkhaniha[3], Yavar Rassi[1], Alireza Zahraei-Ramazani[1], Mohammad Hossein Arandian[4], Reza Jafari[4], Yamdeu Joseph Hubert Galani[5,6], Alireza Sanei-Dehkordi[7], *Mohammad Reza Yaghoobi-Ershadi[1],* Amir Ahmad Akhavan[1,2]

[1]Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Environmental Chemical Pollutants and Pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Human Ecology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[4]Esfahan Research Station, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[5]School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK
[6]Section of Natural and Applied Sciences, School of Psychology and Life Sciences, Canterbury Christ Church University, Canterbury, CT1 1QU, UK
[7]Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

***Corresponding author:** Dr Mohammad Reza Yaghoobi-Ershadi, E-mail: yaghoobi.reza@gmail.com, Dr Amir Ahmad Akhavan, E-mail: aaakhavan@tums.ac.ir

(Received 29 June 2021; accepted 1 November 2021)

Abstract

Background: Among neglected zoonotic diseases, leishmaniases caused by *Leishmania* parasite through infected female sand fly bite, are a group of diseases found in 98 countries and territories representing a critical burden of disease worldwide. Vector management plays a crucial role in reducing the burden of vector-borne diseases by WHO's global plan. The objective of the current study was to assess the susceptibility status of wild phlebotomine sand flies from Esfahan Province, central Iran, to the recommended insecticides by WHO.

Methods: Sand flies were collected by mouth aspirator in Matin Abad desert Eco-resort and were tested using WHO adult mosquito test kit against Dichlorodiphenyltrichloroethane **(**DDT) 4%, Deltamethrin 0.05%, Malathion 5% and Propoxur 0.1%. The number of knockdown sand flies were recorded during exposure time in ten minutes interval for DDT and Deltamethrin and they were allowed to recover for 24 hours. Knockdown $Time_{50}$ (KD_{50}) and KD_{90} were generated for them using Probit software. They were mounted and identified by valid keys.

Results: Among the tested insecticides against female *Phlebotomus papatasi*, DDT, Deltamethrin, and Malathion recorded the highest mortality rate of 100%, followed by Propoxur with 92.2% mortality for a one-hour exposure. For DDT, KD_{50} and KD_{90} were calculated 21.87 and 42.93 and for Deltamethrin, they were 23.74 and 56.50 minutes respectively. Total sand flies exposed with DDT and Deltamethrin shed their leg(s).

Conclusion: It is concluded that *Ph. papatasi* from central Iran is susceptible to DDT, Deltamethrin, Malathion, and Propoxur.

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

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

Introduction

Leishmaniases caused by parasite (Protozoan) are a group of neglected zoonotic diseases (NZDs) that draw more attention among all the neglected tropical diseases (NTDs). There are over 20 *Leishmania* species that are transmitted by the female phlebotomine sand flies through infected bite; a total of 98 sand fly species are identified as medically important vectors (1– 3). The most common form of the disease is cutaneous leishmaniasis (CL) that causes on the exposed parts of the body skin lesion/s mostly ulcer/s and long-life scares (2). Although CL is a self-healing form of the disease, it creates permanent scars and serious disability (4). Approximately 95 % of CL cases occur in the Middle East Mediterranean basin in the old world, and central Asia and the Americas in the new world, and 70% of worldwide cases are related to the Eastern Mediterranean region (2). In 2018 it was reported that 85 % of cases occurred in 10 countries including Iran (2). According to the 2018 WHO report, 98 countries and territories are endemic for leishmaniasis (5). More than 200,000 new cases reported in 2018 and the disability adjusted life years (DALYs) were about 260,000 in 2017(6). Also CL is one of the skin NTDs affecting subcutaneous tissue and skin resulting in disfigurement, disability, stigmatization, and other socio-economic problems (7).

In Iran, leishmaniasis is endemic in many rural areas of 18 provinces out of 31 (CDC, Ministry of Health and Medical Education, Iran, Unpublished data) in the way that several research groups have worked on different aspects of the disease. In addition, some international courses about the disease and its control were conducted which attracted lots of interest among different countries (8–21). *Phlebotomus papatasi* is the first line incriminated vector of zoonotic cutaneous leishmaniasis (ZCL) in Iran. Studies have shown there are 48 species of sand flies, among which 30 species belong to the genus *Phlebotomus*, and 18 species of the genus *Sergentomyia*. Four species of the family Cricetidae of rodents are considered as the main reservoir host including *Rhombomys opimus*, *Meriones libycus*, *Tatera indica*, and *Meriones hurrianae* in different parts of Iran (22).

Diseases transmitted by vectors cause a critical burden in the world, especially in tropical and neotropical areas. Several important vector-borne diseases as parts of NTDs or Skin NTDs in public health continue to need to intensify vector control interventions aimed at monitoring and reducing transmission. The WHO has several global plans to combat NTDs for decades by the multi-intervention packages including integrated vector management (IVM) (23–28). Vector control has a vital role to play in reducing the burden of vector-borne diseases. However, vector control also has proven well-known weaknesses, including the development of insecticide resistance in vectors that played a critical role in the breakdown of the eradication, elimination, and even controlling. Today, there is a need to learn how to monitor and manage vector resistance in a better way (23). Control methods include insecticide spray, use of insecticide-treated nets, environmental management, and personal protection (2). Residual spraying for endophilc, exophilic, and peridomestic sand flies is recommended by World Health Organization Pesticides Evaluation Scheme (WHOPES). Various insecticide classes can be used for indoor residual spraying (IRS), such as organochlorines (for example DDT), synthetic Pyrethroids (for instance Deltamethrin and Lambda-cyhalothrin), organophosphates (for example Malathion), and carbamates (for example Propoxur) (29).

Although major scientific breakthroughs have been made worldwide during recent decades in the different aspects of leishmaniases diagnosis, prevention, treatment and control, morbidity and mortality of that disease still show a worrying raising trend (29). Vector control with insecticides remains one of the most efficient approach to tackle the disease, and targeting

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

adult insects with insecticide compounds has shown good results on the spread of the disease. However, non-managed application of insecticide as harmful poisons in any way can result in long or short term toxicity. It is therefore critical to assess the susceptibility or resistance of vectors against the current or foreseen used insecticides. Toxicity cannot be defined as a single molecular event, it is a cascade of events that start with "Exposure". It proceeds through "distribution and metabolism" and ends with "interaction with cellular macromolecules" and expresses with" toxic endpoint" (30).

Insecticides susceptibility testing has a long history worldwide. Wood (1962) tested *Aedes aegypti* against DDT 1% and 2% and Dieldrin 0.1% and found females more tolerant than males, Pener and Wiliamovsky (1987) tested *Ph. papatasi*, a colony originating from the Jordan Valley, against DDT, Permethrin, and Methoxychlor. They found sand flies susceptible to DDT and Permethrin but tolerant to Methoxychlor, El-Sayed, et al (1989) worked on baseline susceptibility of *Ph. papatasi* and mechanism of resistance by comparing the process with DDT-resistant and susceptible strains of *Culex quinquefasciatus* and *Anopheles gambiae* (31– 33).

The susceptibility investigation on sand flies is less explored due to lack of specific protocol, and researchers who studied it had to follow the test procedure of mosquitoes. In this study, the susceptibility of sand flies as the main vector of ZCL in Iran was tested against DDT, Deltamethrin, Malathion, and Propoxur, following the commonly used mosquitos' protocol, to pave the way towards further studies on sand fly susceptibility with a specific protocol.

Materials and Methods

Study area

This study was conducted in Matinabad Desert Eco-Resort, Matinabad Village, Badroud Rural District, Natanz County (33.753584 N, 51.990596 E), located 60 Km southeastern of Kashan City, Esfahan Province, central Iran (Fig. 1). This area is one of the most important endemic focus of ZCL and one of the most popular touristic desert Eco-resort in Iran which received the peace and environment award of 2015 (34). The average annual rainfall was 15.44 mm, the average temperature was between 11.3 to 21.3 °C, and relative humidity reported

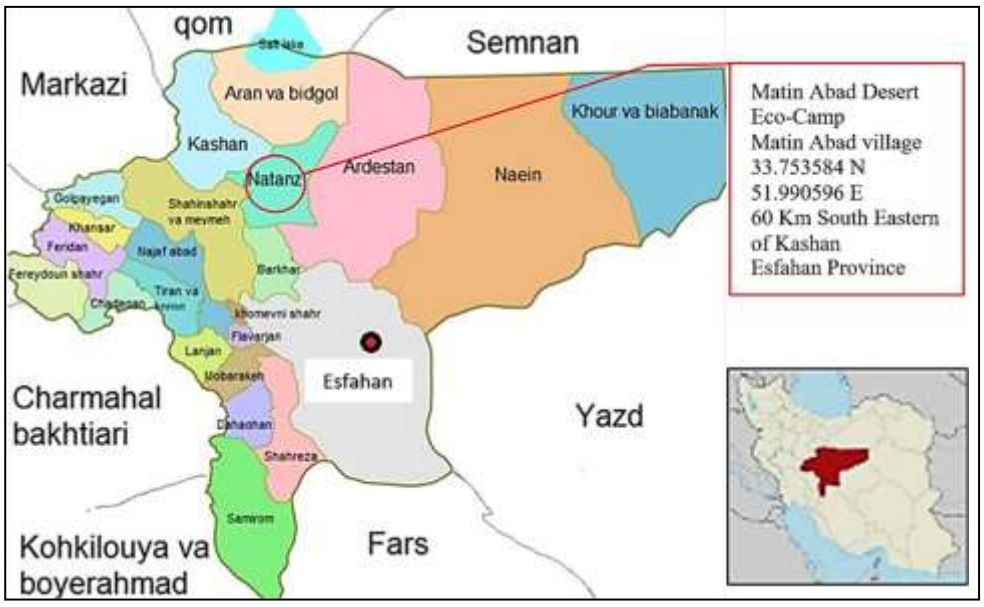

Fig. 1. Map of study area, Esfahan Province, Iran

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

between 30.3–50.7% (Meteorological Organization - Esfahan Province).

Sand fly collection

Sand flies were collected using a filtered mouth aspirator, most of them on a car trap inside Matinabad Desert Eco-Camp before sunset till early in the next morning around *Haloxylon* bushes and rodent burrows, from July to September 2019. Sand flies were kept in the cage with a wet towel and were transported to the sand fly insectary in Esfahan Health Station. Susceptibility tests were conducted the day after in the laboratory. Sand flies were fed with cotton soaked in 10% sucrose solution, and the insects were kept at 25–28 ºC temperature, 70–90% relative humidity, and 14:10 L:D photoperiod.

Insecticides

All WHO test- kit tubes and impregnated papers were procured by CDC, Ministry of Health and Medical Education, Iran by the WHO collaborating center in University Sains Malaysia, Penang, Malaysia. The choice of insecticides was based on highly recommended WHO insecticides at least one from each class such as Organochlorine: Dichlorodiphenyltrichloroethane (DDT) 4% (BATCH No: DD 265), Organophosphate: Malathion 5% (BATCH No: MA 234), Carbamate: Propoxur 0.1% (BATCH No: PR 123) and Pyrethroid: Deltamethrin 0.05% (BATCH No: DE 527).

Bioassay (susceptibility) tests

Since there is no integrated standard protocol for susceptibility testing of sand flies, they were tested using adult susceptibility test procedures of adult mosquitoes based on WHO the latest protocol of 2018. (28)

The WHO susceptibility tube test is a kind of "direct response-to-exposure" test. It measures mosquito mortality to a known standard concentration of a given insecticide, either with a discriminating concentration or with intensity concentrations.(28) Control papers were prepared using 'acetone and silicone oil'-impregnated paper (0.66 ml oil + 1.34 ml acetone) as a control for DDT and Pyrethroid group and 'acetone and olive oil'-impregnated paper (0.71 ml oil + 1.29 ml acetone) as a control for Organophosphate and Carbamates according to the standard method of World Health Organization Pesticides Evaluation Scheme (WHOPES) Institute of Research for Development (IRD), Montpellier, France.

Standard procedure

Sand flies were offered a 10 % sucrose solution for water and energy sources and kept in insectary condition, then transferred to the tubes about one hour prior to starting the test. Insecticide impregnated papers inside test tubes kept refrigerated in a plastic bag were put at room temperature about 1 hour prior to the test. All sand flies were exposed to insecticides for one-hour paralleling with control tubes for each replication. At the end of exposure time, all tubes were kept in insectary condition (T: 25–28 °C- RH: 70–90%) for 24 hours to recover after exposure, with a cotton pad containing 10% sucrose on the top net. Then the mortality of sand flies in both test and control tubes was read and recorded the day after (28).

All sand flies that had the ability to fly were considered alive, regardless of leg losing. The number of knocked down sand flies was recorded every 10 minutes for sand flies exposed to DDT and Deltamethrin. If observed mortality in control groups after 24 h recovery time ranged between 5 to 20%, mortality in the test tubes of that group should be corrected using Abbott's formula (35). If observed mortalities in control groups exceeded 20%, the entire tubes of that group were discarded. For mortality percentage calculation and correction of mortality the following formulas, adopted from WHO (2016) were used (28).

$$\text{Observed mortality} = \frac{\text{Total number of dead sand flies}}{\text{Total sample size}} * 100$$

$$\text{Corrected mortality} = \frac{(\%\ \text{observed mortality} - \%\ \text{control mortality})}{(100 - \%\ \text{control mortality})} * 100$$

Based on the 2018 WHO test procedure

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

if the mortality recorded equal or more than 98%, the tested group will be categorized as susceptible; if the mortality ranged between 90 to 97% it shows the resistance possibility. When it happens, the test must be repeated. If the second test mortality is less than 98% the resistance is confirmed. If the mortality recorded less than 90%, we are facing a confirmed resistance. Then researchers can determine the intensity of resistance or mechanism of resistance by applying following the protocol (28).

Sand flies testing

Total number of 1316 unfed female *Ph. papatasi* sand flies have been tested. Since they were wild, all fed, gravid, semi-gravid females, all males, and other species were excluded at the time of transferring to the test tubes, checking mortality, mounting and also during identification.

Susceptibility tests were carried out on six to fifteen replications in several rounds to obtain enough sand flies tested (at least 100 for each insecticide) with relevant enough control tube/s in each group in parallel.

Sand fly mounting and identification

All sand flies tested after recording the mortality results, transferred to ethanol 70% for mounting and identification. They were mounted in Pouri's media and mounted sand flies' species were identified based on valid identification keys (36, 37).

Knockdown effect and leg loss

The number of knocked down sand flies was counted in the DDT and Deltamethrin test tubes and recorded every ten minutes during the exposure time. Sand flies leg loss was investigated and recorded after 24h recovery in males and females.

Data analysis/ Knockdown curve

The knock down time regression line was created for DDT and Deltamethrin using Probit software and data analysis was made with 95% confidence interval and the KD_{50} and KD_{90} were calculated (Table 1, Fig 3,4) (38) Number of sand flies tested shows in Table 2.

Results

Knock down Time$_{50}$ (KD_{50}) and KD_{90}

The number and percent of knock down sand flies are shown in Fig. 2 and 3. The Probit parameters and the KD_{50} and KD_{90} with 95% confidence interval were calculated (Table 1).

Table 1. The Parameters of Probit regression line of knockdown times for wild-caught sand flies Matinabad desert Eco-resort, Esfahan Province, 2019

Insecticide Name	A	B ± SE	KD_{50}, (LCL-UCL) 95% C.I.	KD_{90}, (LCL-UCL) 95% C.I.	X^2 (df)	P value
Deltamethrin 0.05%	-4.68	3.4 ± 0.326	19.9 **23.74** 27.44	46.75 **56.5** 75.43	12.93 (4)	<0.05
DDT 4%	-5.86	4.38 ± 0.495	17.74 **21.87** 25.69	35.81 **42.93** 56.84	23.78 (4)	<0.05

A = y-intercept
B = the slope of the line;
SE = Standard error;
KD_{50}, 95 % CI = Time causing 50 % Knockdown and its 95 % confidence interval
KDT_{90}, 95 % CI = Time causing 90 % Knockdown and its 95 % confidence interval
LCL: Lower Confidence Limit
UCL: Upper Confidence Limit
X^2 = Heterogeneity about the regression line
df = degree of freedom
P value = Represent heterogeneity in the population of tested

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

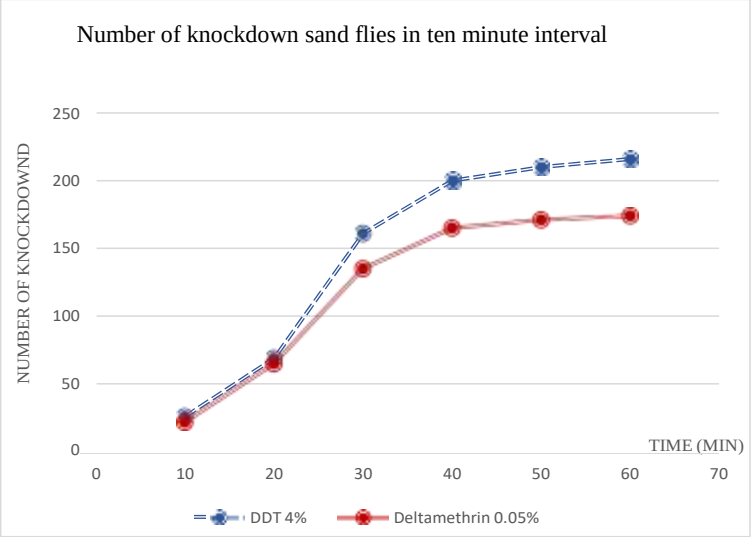

Fig. 2. The number of knockdown sand flies in ten-minute intervals during exposure time with DDT and Deltamethrin. Matinabad desert Eco-resort, Esfahan Province, 2019

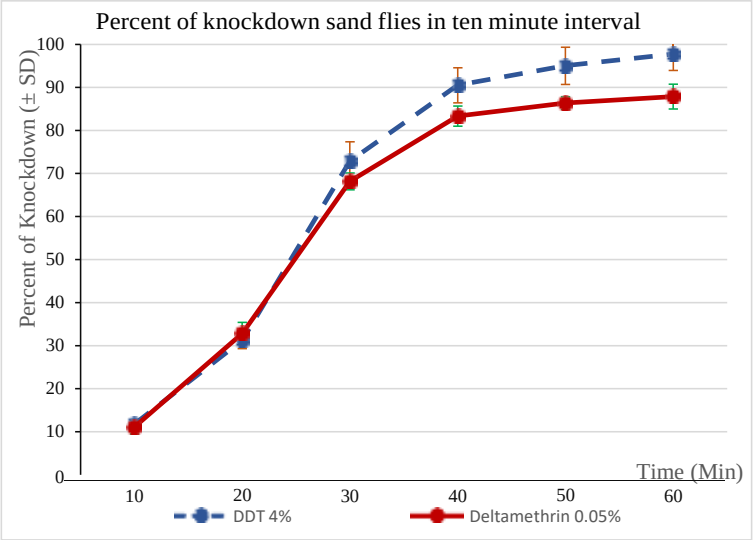

Fig. 3. Knockdown percent of sand flies exposed to DDT and Deltamethrin for one hour in ten-minute intervals. Matinabad desert Eco-resort, Esfahan Province, 2019

Total sand flies exposed with DDT and Deltamethrin shed their leg(s).

Additionally, sand flies exposed to these two insecticides experienced the "knockdown effect" evidently by muscle spasm, involuntary movement/move less (convulsion or erratic movement or paralysis) during the exposure time (39). It was observed that sand flies exposed with DDT had more involuntary movements and then the ones exposed with Deltamethrin who were more moveless.

Susceptibility status

The susceptibility status of female sand flies is shown in Table 2. The mortality rate of sand flies exposed to Propoxur has shown a possible resistance in the first round of test and according to the most recent test protocol, the test was repeated in 2 more

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

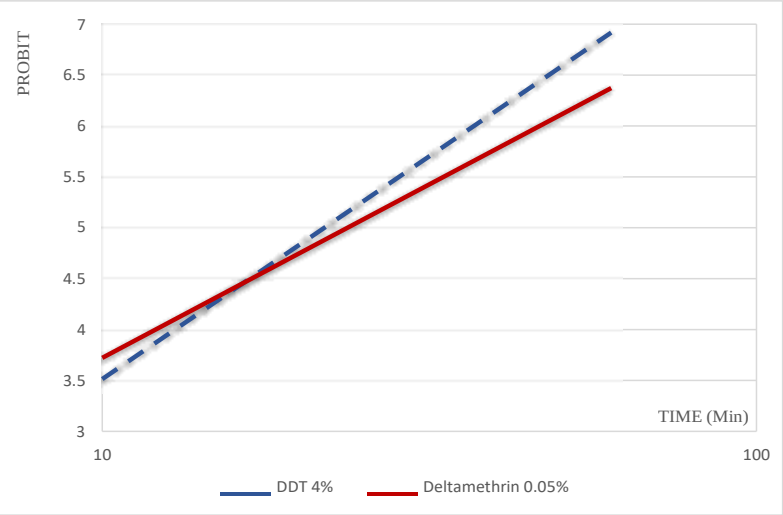

Fig. 4. The regression line for DDT and Deltamethrin for sand flies exposed for one-hour. Matinabad desert Eco-resort, Esfahan Province, 2019

Table 2. Susceptibility status of female *Phlebotomus papatasi* to different insecticides- Matinabad desert Eco-resort, Esfahan Province, 2019

Insecticide/ Concentration	Test			Control			Result
	Total No. of unfed females	No. of dead	Mortality rate (%)	Total No. of unfed females	No. of dead	Mortality rate (%)	Susceptibility status
DDT 4%	134	134	100	174	2	1.1	Susceptible
Deltamethrin 0.05%	138	138	100	109	1	0.9	Susceptible
Malathion 5%	223	223	100	75	1	1.3	Susceptible
Propoxur 0.1% 1st round	105	95	90.47	75	1	1.3	
Propoxur 0.1% 2nd round	133	132	99.24	82	1	1.2	Susceptible

rounds and replications obtaining enough number sand flies.

In the current study, 1248 female *Ph. papatasi* were exposed to different standard discriminative concentrations of insecticides. One hundred and thirty-four unfed adult *Ph. papatasi* were exposed to DDT 4% which resulted in 100% mortality, showing that this species is susceptible to DDT insecticide. For Deltamethrin 0.05% and Malathion 5%, 138 and 223 females were tested respectively, and both of them resulted in 100% mortality that was determined as a totally susceptible population. One hundred and five sand flies were tested against Propoxur 0.1% and resulted in 90.4% mortality which was shown as resistance at the first glance. According to the 2018 WHO guideline, the second round of tests was done using 133 females and resulted in 99.2% mortality that was evaluated as susceptible species. While control groups were tested in parallel for each batch accordingly by recorded mortality of 1.1 and 0.9% for acetone/ silicone oil and 1.3% and 1.2% for acetone/ olive oil respectively.

Survival curve

Regression analysis was performed for *Ph. papatasi* to estimate KD_{50} and KD_{90}

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

for DDT and Deltamethrin with a 95% confidence interval. A knockdown time regression line was created for them showed in Fig. 4.

Discussion

The objective of the current research was to investigate the susceptibility/resistance of *Ph. papatasi* to certain insecticides from various chemical classes using the WHO test kit. World Health Organization bioassay susceptibility test kit is a direct response-to-exposure test that is vital in insecticide resistance management worldwide (24). The discovery of DDT in 1939 was one of the most meaningful developments in the history of pest control. Deltamethrin also was the most active insecticide ever known at the time of its discovery. Continuous use of many insecticides is a potential threat in the field of emerging resistance in insects (40).

Wild-caught *Ph. papatasi* in the current study was found to be susceptible to Organochlorine(DDT4%),Organophosphate (Malathion 5%), Pyrethroid (Deltamethrin 0.05%), and Carbamate (Propoxur 0.1%). There are studies conducted worldwide on baseline susceptibility of various species of sand flies in different countries.

Old world

In India *Phlebotomus argentipes* reported resistant to DDT in 1992 and in different parts of Bihar they found developing resistance to DDT 4% in 2001. (41, 42) In the latter study they do their research on a different species from our study. In North Africa and the Middle East researchers worked on Bendiocarb, Cyfluthrin, DDT, Malathion, Permethrin, and Resmethrin on *Phlebotomus bergeroti*, *Phlebotomus langeroni*, *Ph. papatasi* and *Phlebotomus sergenti* in 2001. They worked on four different species from ours and various insecticide and reported 3 least toxic insecticides in order of toxicity Permethrin, Malathion and DDT, with DDT being the least toxic. It has been stated that the response to three other insecticides: Bendiocarb, Cyfluthrin and Resmethrin has not been as uniform among species (43). In Italy (2002) *Phlebotomus perniciosus* and *Ph. papatasi* were susceptible to DDT 2%, Lambda-cyhalothrin 0.06% and Permethrin 0.2% (44). It agree with our study while the percentage of DDT is less than current experiment. In some parts of India and Nepal border *Ph. argentipes* in 2010–2012 reported resistant to DDT 4% and susceptible to Deltamethrin 0.05% and Malathion 5%. They conducted the study on different species and their result about DDT was not same as ours. It is explained that the use of DDT in IRS measures for VL control could effect on sand flies susceptibility (45, 46). Also, in 2012 *Ph. papatasi* and *Ph. sergenti* were susceptible to DDT and Lambda-cyhalothrin in Morroco, in parallel with our report (47). In some area of Sudan populations of *Ph. papatasi* was susceptible to DDT, Permethrin, Malathion, and Propoxur that is agree to our research and in some area sensitive to DDT and Permethrin but highly resistant to Malathion and Propoxur in 2012 which is probably due to anti-malaria activities during last 50 years in the area and it is on the contrary to our experiments (48). In 2015 *Ph. argentipes* in West Bengal, India reported developing resistance to DDT (49). In Kerala *Ph. argentipes*, *Ph. sintoni*, *Sergentomyia bagdhadis*, *Se. zeylonica* and *Se. babu* were susceptible to DDT and Deltamethrin (50). *Phlebotomus argentipes* in 2016 reported resistance in Kala-azar endemic region and susceptible to DDT in the non-endemic region in India (51). All of these last-mentioned studies have been done by different sand flies species from ours. In two different Western provinces of Turkey with and without a background of insecticides use, populations of sand flies found resistant and susceptible to Deltamethrin 0.05% and Permethrin 0.75% respectively (2017) as a result of long term application of both insecticides in the region (52). They did not mention the species of sand flies tested. In 13 villages of Bihar *Ph. argentipes* as a different species from our region was highly susceptible to Deltamethrin,

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

Lambda-cyhalothrin, Alpha-cypermethrin (2016) (53). In Nepal and Bangladesh *Ph. argentipes* was highly susceptible to Alpha-cypermethrin 0.05%, Deltamethrin 0.05%, Lambda-cyhalothrin 0.05%, Permethrin 0.75%, Malathion 5% and Bendiocarb 0.1% in 60 min of exposure (2017) (54). In the last two studies they tested *Ph. argentipes* that this species is a vector on that area but not in Iran.

Also in Iran, there are studies on the susceptibility status of sand flies. During 1985–88 Seyedi Rashti et al experimented on various areas of Iran with the treatment background with DDT which discontinued from 1969. They expressed that sand flies from Esfahan showed more tolerance against DDT in comparison to other areas. (8) But our experiments show different condition in this area now. Yaghoobi Ershadi and Javadian found *Ph. papatasi* tolerant to DDT 4% in Borkhar County in Esfahan Province due to DDT or related compound application in public health or in agricultural pest control which is in contrary to our results, but susceptible to Dieldrin 4% and in Varzane they were susceptible to DDT 4% similar to our research results (9, 10). It is reported that *Ph. papatasi* and *Ph. sergenti* was susceptible to DDT 4% in Kerman province. (11). It agree with our result about *Ph. papatasi*. In 1998 a study showed that *Ph. kandelakii* and *Ph. perfiliewi* as a probable vector of zoonotic visceral leishmaniasis (ZVL) were susceptible to DDT 4% during 1994 in Ardabil province, Northwest of Iran (12). These species are in different area where it is a ZVL foci with different vectors from ZCL. In Arsanjan County of Fars Province, *Ph. papatasi* recorded sensitive to DDT 4% in 1999 same as current report (13). It is showed that *Ph. sergenti* was susceptible to DDT 4% in Esfahan city in 2005 it is a study on a different species in same province (14). In 2004 and 2005 in Bam City, Kerman Province *Ph. papatasi* and *Ph. sergenti* were susceptible to DDT 4% and Deltamethrin 0.05% similar to this reports (15). Wild-caught *Ph. papatasi* in Badrood, Esfahan Province and their progeny were found susceptible to Permethrin 0.75%, Deltamethrin 0.1%, Cyfluthrin 0.15% and Lambda-cyhalothrin 0.05% and to DDT 4% During summer 2010 (16, 17). Our research also confirm the susceptibility of them in this area to DDT and Deltamethrin. Another study in the same place during summer 2015 showed that there is susceptibility to Cyfluthrin 0.15%, Lambda-cyhalothrin 0.05%, Permethrin 0.75%, and Deltamethrin 0.05% same as our report and tolerant to DDT 4% unlike to our study (18). A study in North Khorasan showed the development of resistance against DDT (4%) in the wild strain of *Ph. sergenti* but susceptible to Bendiocarb 0.1% and Permethrin 0.75% (19). This report is about another species with various insecticide differ from our experiment. During 2016 and 17 Laboratory reared of *Ph. papatasi* were found susceptible to Permethrin 0.75%, Deltamethrin 0.05%, Cyfluthrin 0.15%, and Lambda-cyhalothrin 0.05% but resistant candidate to DDT 4% (20). This study reported likely result about Deltamethrin and unlike result from current research about DDT.

New world

In 1997 a comprehensive study carried out on field population of *Lutzomyia longipalpis* of Venezuela against DDT 2%, Propoxur 0.01 %, Malathion 2%, Fenitrothion l%, Pirimiphos methyl l%, Deltamethrin 0.06%, Lambda-cyhalothrin 0.06%, and permethrin 0.2% insecticides and compered with laboratory population of reference strain and reported highly susceptible (55). The species used in this experiment is different from ours because in new world *Lu. longipalpis* has medical importance as a vector but there is no in the old world and the concentration of Deltamethrin, Malathion and DDT used in their study are not same as concentration used in current study. In 2009 researchers reported two wild populations of *Lu. longipalpis* with different exposure backgrounds susceptible to Malathion, Fenitrothion, Lambda-cyhalothrin, Permethrin, and Deltamethrin in Brazil (56). In 2015 another study in Brazil reported *Lu. longipalpis* highly

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

susceptible to Alpha-cypermethrin (57). Also Brazil located in new world and the vector is *Lu. Longipalpis* and the only common insecticide was Deltamethrin. In the United State, some tests performed on laboratory populations of *Ph. papatasi* and *Lu. longipalpis* using CDC bottle bioassay against different concentrations of Cypermethrin, Deltamethrin, Lambda-cyhalothrin and Permethrin, Chlorpyrifos, Fenitrothion, and Malathion, Bendiocarb, Propoxur and DDT and they documented as susceptible population (39). Same species and same insecticide tested by different methods of CDC bottle bioassay but reported the same result. In Colombia in a study with the same method on *Lu. longipalpis*, Lambda-cyhalothrin showed the highest degree of toxicity followed by Alpha-cypermethrin and Deltamethrin (58). There is another study in Brazil using a modified method of WHO comparing laboratory population of *Lu. longipalpis* with some population in the field with different exposure background and reported that Lab-reared sand flies were more tolerant to field-collected ones against Lambda-cyhalothrin (0.05%), Deltamethrin (0.5%) and control was (Silicone oil) (59). The sand fly species is different and also the concentration of Deltamethrin is not the same.

It can be observed that the only resistant *Phlebotomus* registered in The Arthropod Pesticide Resistance Database is *Ph. argentipes* in 23 locations of Bihar state in India (60– 62). It is reported as resistance to DDT in VL endemic area of Bihar and also developed resistance/ tolerant to Malathion in a larger area but susceptible to Deltamethrin and the wild-caught and their seven offspring's is reported resistant to DDT (60, 61). They also experimented another species in different location and the result also is unlike to current research.

In the current study, it was found that sand flies from Esfahan Province, were highly susceptible to Deltamethrin and DDT and it was also noted that during the exposure time and counting the knockdown numbers of sand flies, those who exposed with DDT had more involuntary movement in their place but the vast majority of those who exposed to Deltamethrin was moveless. Pyrethroids as a major class of neurotoxic insecticide and DDT, fairly slow-acting on the protein of voltage-gated sodium channels in the cell membrane of the insect nerves. Exposing insects to DDT and Deltamethrin disrupts the normal process leading to paralysis and finally death. Peripheral nervous system influenced by DDT causing tremors in appendages or entire body called "DDT Jitters" then leads to excitatory paralysis and eventually death. Deltamethrin affects both the central and peripheral nervous systems by producing repetitive discharge and cause paralysis the same as DDT but more obvious. After exposure with Deltamethrin, the channels remain open and leads to abnormal hyperexcitability but "Knockdown" is its sub-lethal effect (40).

Sand flies in response to exposure to DDT and Deltamethrin manifested evident leg shedding in the current study. The same observation was made by Denlinger and Alexander (39, 56). Sand flies with shedding legs, as a significant sub-lethal effect, will not be able to transmit the parasite as a consequence of disabling for blood-feeding (56). On the other hand, the authors reported that sand flies after shedding legs could still be capable of blood feed (39). We did not check the ability to have blood meals for leg shedded sand flies because the mortality rate was high, they were wild-caught, and we needed to identify them after keeping in alcohol and mounting. Nevertheless, this will be considered in further studies.

Conclusion

This study revealed that *Ph. papatasi* from central Iran is susceptible to DDT, Deltamethrin, Malathion, and Propoxur. Knowing about the susceptibility/ resistance of sand flies in this endemic area can play a vital role in the field of vector control and pesticide management. Excessive use of insecticide with unsuitable concentration can cause resistance in vector sand flies and complicate

http://jad.tums.ac.ir
Published Online: Dec 31, 2021

disease control. This result brings additional data to the worldwide need to assess the insecticide susceptibility status of sand flies, in order to strengthen vector surveillance and integrated vector management. We strongly recommend performing susceptibility tests on sand flies in various parts of the world as systematic monitoring and evaluating the status of leishmaniasis vectors against various insecticides, as regular or periodic susceptibility tests can ring a timely alert regarding early resistance. Also doing some further tests on the resistant ones is recommended to determine the resistance intensity and mechanism according to standard protocols of WHO.

Acknowledgements

The authors would like to appreciate Dr Nikpoor at TUMS for procurements of the susceptibility test kit, and standard impregnated filter papers. We also appreciate Professor Corbel and his team at IRD, Montpellier, France for their kind support and for generous sharing and training the Impregnation methods and principles. Many thanks to the personnel and manager of Matinabad Desert Eco-resort particularly Mr SH Vaghefi for their precious cooperation and kind support.

This study was conducted as a part of a PhD dissertation of Zahra Saeidi, by financial supports of School of Public Health (SPH) and Institute of Environmental Research (IER), Tehran University of Medical Sciences (TUMS), code of 96-04-27-35872.

Ethical considerations

This study was conducted as a part of a PhD dissertation, and It has been reviewed and approved by the School of Public Health (SPH), Tehran University of Medical Sciences (TUMS) ethics committee and has been registered with the code IR.TUMS. SPH.REC.1396.3602.

Conflict of interest statement

Authors declare that there is no conflict of interest.

References

1. World Health Organization (WHO), (2020) Neglected diseases, available at https://www.who.int/neglected_diseases/zoonoses/infections_more/en/ (access 20 march 2020)
2. World Health Organization (WHO), (2020) Leishmaniases fact sheet, available at https://www.who.int/news-room/factsheets/detail/leishmaniasis - (access 20 march 2020)
3. World Health Organization (WHO), (2020) Leishmaniasis interactive timelines available at https://www.who.int/leishmaniasis/disease/Leishmaniasis-interactive-timelines/en/ (access 20 march 2020)
4. World Health Organization (WHO), (2020) resources and publication available at https://www.who.int/csr/resources/publications/CSR_ISR _2000_1leish/en/ (access 20 march 2020)
5. World Health Organization (WHO), (2020) Neglected diseases, Leishmaniasis available at https://www.who.int/gho/neglected_diseases / leishmaniasis/en/ (access 20 march 2020)
6. World Health Organization (WHO) (2020) Ending the neglect to attain the Sustainable Development Goals – A road map for neglected tropical diseases 2021–2030. World Health Organization; annex 2Geneva..
7. World Health Organization (WHO) (2020) Ending the neglect to attain the Sustainable Development Goals – A road map for neglected tropical diseases 2021–2030. World Health Organization; Licence: CC BY-NC-SA 3.0 IGO, Geneva..
8. Seyedi-Rashti MA, Yezdan-panah H, Shah Mohamadi H, Jedari M **(**1992) Susceptibility of *Phlebotomus papatasi* (Diptera: Psychodidae) to DDT in some foci of cutaneous leishmaniasis in Iran. J Am Mosq Control Assoc. 8(1): 99–100.
9. Yaghoobi-Erashadi MR, Javadian E **(**1995) Susceptibility status of *Phelebotomus papatasi* to DDT in the most important focus of zoonotic cutaneous leishmaniasis, Esfahan Province, Iran. Iran J Public Health. 24: 11–19.
10. Yaghoobi-Ershadi MR, Javadian E **(**1993) Susceptibility of *Phlebotomus papatasi* to DDT in the most important focus of zoonotic cutaneous leishmaniasis, Isfahan Province, Iran. J Entomol Soc Iran . 12: 27–37 (in Persian).
11. Aghasi M (1996) Present status of anthroponotic cutaneous leishmaniasis in Kerman, southeast Iran [MSPH thesis] School of Public Health, Tehran University of Medical Sciences, Iran (in Persian).
12. Rassi Y, Javadian E **(**1998) The susceptibility to 4% DDT and host preference of the probable vectors of visceral leishmaniasis in northwest of

Iran. Iran J Public Health. 27(1-2): 47–54.
13. Rassi Y, Jalali M, Vatandoost H **(**2000) Susceptibility status of *Phlebotomus papatasi* to DDT in Arsanjan County in Fars Province, Iran. Iran J Public Health. 29(1-4): 21–26.
14. Zahraei Ramezani AR, Yaghoobi- Ershadi MR, Akhavan AA, Abdoli H, Jafari R, Jalali Zand AR, Arandian MH, Shareghi N and Ghanei M (2008) Some ecological aspect of phlebotomine sand flies (Diptera: Pchychodidae) in an endemic focus of ACL of Iran. J Entomol. 5(1): 17–23.
15. Afshar AA, Rassi Y, Sharifi I, Abai MR, Oshaghi MA, Yaghoobi-Ershadi MR, Vatandoost H (2011) Susceptibility status of *Phlebotomus papatasi* and *P. sergenti* (Diptera: Psychodidae) to DDT and Deltamethrin in a focus of cutaneous leishmaniasis after earthquake strike in Bam, Iran. Iran J Arthropod Borne Dis. 5: 32–41.
16. Saeidi Z, Vatandoost H, Akhavan AA, Yaghoobi-Ershadi MR, Rassi Y, Sheikh Z, Arandian MH, Jafari R, Sanei-Dehkordi AR (2012) Baseline susceptibility of a wild strain of *Phlebotomus papatasi* (Diptera: Psychodidae) to DDT and Pyrethroids in an endemic focus of zoonotic cutaneous leishmaniasis in Iran. Pest Manag Sci. 68(5): 669–675.
17. Saeidi Z, Vatandoost H, Akhavan AA, Yaghoobi-Ershadi MR, Rassi Y, Arandian MH, Jafari R (2013) Baseline insecticide susceptibility data of *Phlebotomus papatasi* in Iran, J Vector Borne Dis. 50: 57–61.
18. Shirani-Bidabadi L, Zahraei-Ramazani AR, Yaghoobi–Ershadi MR, Rassi Y, Akhavan AA, Oshaghi MA, Enayati AA, Saeidi Z, Jafari R, Vatandoost H (2017) Assessing the insecticide susceptibility status of field population of *Phlebotomus papatasi* (Diptera: Psychodidae) in a hyperendemic area of zoonotic cutaneous leishmaniasis in Esfahan Province, Central Iran, Acta Trop. 176: 316–322.
19. Arzamani K, Vatandoost H, Rassi Y, Abai MR, Akhavan AA, Alavinia M, Akbarzadeh K, Mohebali M, Rafizadeh S (2017) Susceptibility status of wild population of *Phlebotomus sergenti* (Diptera: Psychodidae) to different imagicides in a endemic focus of cutaneous leishmaniasis in northeast of Iran. J Vector Borne Dis. 54: 282–286.
20. Shirani-Bidabadi L, Zahraei-Ramazani AR, Yaghoobi-Ershadi MR, Akhavan AA, Oshaghi MA, Enayati AA, Rassi Y, Gholampour F, Shareghi N, Madreseh E, Vatandoost H (2020) Monitoring of laboratory reared of *Phlebotomus papatasi* (Diptera: Psychodidae), main vector of zoonotic cutaneous leishmaniasis to different imagicides in hyper endemic areas, Esfahan Province, Iran. J Arthropod Borne Dis. 14(1): 116–125.
21. Yaghoobi-Ershadi MR, Akhavan AA, Shirzadi MR, Rassi Y, Khamesipour A, Hanafi-Bojd AA, Vatandoost H (2019) Conducting international diploma course on leishmaniasis and its control in the Islamic Republic of Iran. J Arthropod Borne Dis. 13(3): 234–242.
22. Yaghoobi-Ershadi MR **(**2016) Control of phlebotomine sand flies in Iran: a review article. J Arthropod Borne Dis. 10(4): 429–444.
23. World Health Organization (WHO) (2008) position statement on integrated vector management, World Health Org. Geneva.
24. World Health Organization (WHO) (2017) Global vector control response 2017–2030. World Health Org. Geneva.
25. World Health Organization (WHO) (2012) Global plan for insecticide resistance management in malaria vectors. World Health Org. Geneva.
26. World Health Organization (WHO) (2013) Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquito. World Health Org, Geneva.
27. World Health Organization (WHO) (2016) Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquito. World Health Org, Geneva .
28. World Health Organization (WHO) (2016) Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquito. 2nd ed. World Health Org. Geneva.
29. World Health Organization (WHO) (2010) 2010 WHO Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, World Health Org Geneva, 22–26.
30. Ernest Hodgson (2004) A textbook of modern toxicology, third edition, Department of Environmental and Biochemical Toxicology. John Wiley & Sons, Inc., New Jersey.
31. Wood RJ **(**1962) The effectiveness of insecticide-impregnated papers of different ages in the WHO adult mosquito test Kit. Bull World Health Organ. Geneva 27: 306.
32. Pener H, Wilamovsky A **(**1987) Base-line susceptibility of *Phlebotomus papatasi* to insecticides. Med Vet Entomol. 1(2):147–149.
33. El-Sayed S, Hemingway J, Lane RP) 1989(Susceptibility baselines for DDT metabolism and related enzyme systems in the sandfly *Phlebotomus papatasi* (Scopoli) (Diptera: Psychodidae), Bull Entomol Res. 79(4) 679– 684.
34. Matinabad Desert Eco-Resort (2020) Website of Matinabad Desert Eco-Resort. Available at: https://matinabad.com/ (accessed 20 March 2020).

35. Abbott WS)1925(A method of computing the effectiveness of an insecticide. J Econ Entomol. 18: 265–267.
36. Seyedi-Rashti MA, Nadim A (1992 (The genus *Phlebotomus* (Diptera: Psychodidae: Phlebotominae) of the countries of the Eastern Mediterranean region. Iran J Public Health. 21(1-4): 11–50.
37. Theodor O, Mesghali A (1964) On the Phlebotomine of Iran. J Med Entomol. 1:285–300.
38. Finney DJ)1971(Probit Analysis, 3rd edition. Cambridge University Press, Cambridge.
39. Denlinger DS, Lozano-Fuentes S, Lawyer PHG, Black IV, WC, Bernhardt SA (2015) Assessing insecticide susceptibility of laboratory *Lutzomyia longipalpis* and *Phlebotomus papatasi* sand flies (Diptera: Psychodidae: Phlebotominae). J Med Entomol. 52(5): 1003–1012.
40. Davies TGE, Field LM, Usherwood PNR, Williamson MS (2007) DDT, pyrethrin, pyrethroids, and insect sodium channels. IUBMB Life. 59: 151–162.
41. Mukhopadhyay AK, Sexena NBLK, Narasimhan MVVL (1992) Susceptibility status of *Phlebotomus argentipes* to DDT in some Kala-azar endemic districts of Bihar, India. Geneva, World Health Organization, (unpublished document WHO/CTD/VBC/ 92.995).
42. Singh R, Das RK, Sharma SK)2001(Resistance of sand flies to DDT in Kala-azar endemic districts of Bihar, India. Bull World Health Organ. 79(8): 793.
43. Gary ET, Abd el-baset BZ, Hanafi AH, Gregory MB, Brian CZ (2001(Susceptibility of sand flies to selected insecticides in North Africa and the Middle East. J Am Mosq Control Assoc. 17 (l): 23–27.
44. Maroli M, Cianchi T, Bianchi R, Khoury C (2002(Testing insecticide susceptibility of *Phlebotomus perniciosus* and *P. papatasi* (Diptera: Psychodidae) in Italy. Ann Ist Super Sanità. 38 (4): 419–423.
45. Dinesh DS, Das ML, Picado A, Roy L, Rijal S, Singh SP 2010 Insecticide susceptibility of *Phlebotomus argentipes* in Visceral Leishmaniasis Endemic Districts in India and Nepal. PLoS Negl Trop Dis. 4(10): e859.
46. Singh RK, Mittal PK, Dhiman RC) 2012(Insecticide susceptibility status of *Phlebotomus argentipes*, a vector of visceral leishmaniasis in different foci in three states of India. J Vector Borne Dis. 49: 254–257.
47. Faraj C, Ouahabi S, Adlaoui EB, El Elkohli M, Lakraa L, El Rhazi M, Ameur B (2012) Insecticide susceptibility status of *Phlebotomus* (*Paraphlebotomus*) *sergenti* and *Phlebotomus* (*Phlebotomus*) *papatasi* in endemic foci of cutaneous leishmaniasis in Morocco. Parasit Vectors. 5: 51–57.
48. Hassan MM, Widaa SO, Osman OM, Numiary MS, Ibrahim MA, Abushama HM (2012(Insecticide resistance in the sand fly, *Phlebotomus papatasi* from Khartoum State, Sudan. Parasit Vectors. 5: 46–55.
49. Kumar V, Shankar L, Kesari S, Shankar Bhunia G, Singh D, Mandal R, Das P (2015) Insecticide susceptibility of *phlebotomus argentipes* & assessment of vector control in two districts of West Bengal, India. Indian J Med Res. 142: 211–215.
50. Selvakumar M, Srinivasan R (2015) Susceptibility status of *Phlebotomus argentipes* to DDT and deltamentrin in a focus of cutaneous leishmaniasis in kani tribes settlement of the Western Ghats in Kerala, India. Int J Curr Res. 7(08): 19564–19566.
51. Dhiman RC, Yadav RS (2016) Insecticide resistance in phlebotomine sandflies in Southeast Asia with emphasis on the Indian subcontinent. Infect Dis Poverty. 5: 106.
52. Nandi J, Chaudhuri I, Das C, Mukhopadhyay AK (2016) Susceptibility Status of *Phlebotomus argentipes*, to Synthetic Pyrethroid Insecticides in Kala Azar Endemic Parts of Bihar, India in Relation to Elimination of Visceral Leishmaniasis. Indian J Public Health Res Dev. 7(4): 36–38.
53. Karakus M, Gocmen B, Özbel Y (2017) Insecticide susceptibility status of wild-caught sand fly populations collected from two leishmaniasis endemic areas in Western Turkey. J Arthropod-Borne Dis. 11(1): 86–94.
54. Chowdhury R , Lal Das M, Chowdhury V, Roy L, Faria S, Priyanka J, Akter S, Prosad Maheswary N, Karim Khan R, Argaw D, Kroeger A (2018) Susceptibility of field-collected *Phlebotomus argentipes* (Diptera: Psychodidae) sand flies from Bangladesh and Nepal to different insecticides. Parasit Vectors. 11: 336.
55. Mazzarri MB, Feliciangeli MD, Maroli M, Hernandez A, Bravo A (1997) Susceptibility of *Lutzomyia longipalpis* (diptera: psychodidae) to selected insecticides in an endemic focus of visceral leishmaniasis in Venezuela. J Am Mosq Control Assoc. 13(4): 335–341.
56. Alexander B, Barros VC, de Souza SF, SS Barros, Teodoro LP, Soares ZR, Gontijo NF, Reithinger R (2009) Susceptibility to chemical insecticides of two Brazilian populations of the visceral leishmaniasis vector *Lutzomyia longipalpis* (Diptera: Psychodidae). Trop Med Int Health. 14: 1272–1277.
57. DÁvila Pessoa GC, Lopes JV, Rocha MF, Pinheiro LC, Luiz Rosa AC, Michalsky EM, Dias ES (2015) Baseline susceptibility to alpha-cypermethrin in

Lutzomyia longipalpis (Lutz & Neiva, 1912) from Lapinha Cave (Brazil). Parasit Vectors. 8: 469.
58. Santamaría E, Marceló C **(**2019) Toxic activity of pyrethroids in *Lutzomyia longipalpis* (Diptera: Psychodidae) from Magdalena River basin, Colombia. Acta Biolo Colomb. 24(2): 391–396.
59. González MA, Bell1MJ, Bernhardt SA, Brazil RP, Dilger E, Courtenay O, Hamilton JGC (2019) Susceptibility of wild-caught *Lutzomyia longipalpis* (Diptera: Psychodidae) sand flies to insecticide after an extended period of exposure in western São Paulo, Brazil. Parasit Vectors. 12: 110.
60. The Arthropod Pesticide Resistance Database, (2020) available at https://www.pesticideresistance.org/display.php?page=species&arId=676, access on 29 December 2020.
61. Singh R, Kumar P (2015) Susceptibility of the Sandfly *Phlebotomus argentipes* Annandale and Brunetti (Diptera: Psychodidae) to Insecticides in Endemic Areas of Visceral Leishmaniasis in Bihar, India. Jpn J Infect Dis. 68: 33–37.
62. Rama A, Kumar V, Kesari S, Singh V, Das P **(**2015) Monitoring Susceptibility Status of *Phlebotomus argentipes* (Diptera: Psychodidae) at Bihar (India) for the Procurement of Homozygous DDT Resistant Colony. J Trop Dis. 3: 4.

http://jad.tums.ac.ir
Published Online: Dec 31, 2021