

Original Article**Insecticide Susceptibility Status of Wild Population of *Phlebotomus kandelakii* and *Phlebotomus perfiliewi transcaucasicus* Collected from Visceral Leishmaniasis Endemic Foci in Northwestern Iran**Yavar Rassi¹; *Eslam Moradi-Asl^{2,3}; *Hassan Vatandoost^{1,4}; Malek Abazari², Abedin Saghafipour⁵¹Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran²Department of Public Health, School of Public Health, Ardabil University of Medical Sciences, Ardabil, Iran³Arthropod Borne Diseases Research Center, Ardabil University of Medical Sciences, Ardabil, Iran⁴Department of Chemical Pollutants and pesticides, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran⁵Department of Public Health, School of Public Health, Qom University of Medical Sciences, Qom, Iran

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Abstract**Background:** *Phlebotomus kandelakii* and *Phlebotomus perfiliewi transcaucasicus* sand flies are the vectors of visceral leishmaniasis in Iran. The aim of this study was to evaluate the susceptibility of *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus*, collected from an endemic focus of visceral leishmaniasis (VL) in the northwest of Iran, to different selected insecticides.**Methods:** Sand flies were collected from the villages of Meshkinshahr and Germe Counties using light traps and aspirators from May to October 2019. The sand flies were identified as *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus* using standard identification keys. Susceptibility test was carried out against DDT (4%), Malathion (5%), Propoxur (0.1%) and Lambda-cyhalothrin (0.05%) according to the WHO standard method. MedCalc statistical software was employed to calculate LT₅₀ and LT₉₀ and to compare the chances of sand flies surviving the exposure to the studied insecticides.**Results:** A total of 1,278 female specimens were used for the susceptibility tests. Out of which 1,063 samples were used for the test and 215 for the control group. The estimated LT₅₀ values for DDT (4%), Malathion (5%), Propoxur (0.1%), and Lambda-cyhalothrin (0.05%) for *Ph. kandelakii* were 15.1, 13.4, 15.4 and 5.8 minutes respectively, and for *Ph. perfiliewi transcaucasicus* were 11.9, 15.6, 15.9 and 5.8 minutes respectively.**Conclusion:** This susceptibility studies revealed different LT₅₀ values for different insecticides efficient against *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus*. The regular monitoring for the resistance of *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus* sand flies seems necessary in diseases control programs in this area.**Keywords:** *Phlebotomus kandelakii*; *Phlebotomus perfiliewi transcaucasicus*; Insecticide resistance; Insecticide susceptibility; Lambda-cyhalothrin**Introduction**

Leishmaniasis is transmitted to human in 98 countries and 3 territories on 5 continents in the world and approximately 0.2 to 0.4 cases of Visceral leishmaniasis (VL) and 0.7 to 1.2 million cases of cutaneous leishmaniasis (CL) occur each year. Leishmaniasis is a parasitic disease found in tropical and subtropical

areas around the world. It has been categorized as a neglected tropical disease (NTD). Leishmaniasis is triggered by infection with *Leishmania* parasites, which spread through the bite of phlebotomine sand flies of *Phlebotomus* and *Lutzomyia*. There are several distinct types of leishmaniasis in humans. The most prevalent

types are CL (causing skin sores) and VL (causing impacts on several internal organs) (1). VL, also known as kala-azar, is a severe health problem in endemic areas. The disease is of great significance to the health care system around the globe and can be fatal without adequate therapy (2). The primary causative agent of VL is *Leishmania infantum* in Mediterranean areas such as Iran (3). In the Mediterranean region, there are two major forms of severe and fatal visceral leishmaniasis and cutaneous leishmaniasis. The worldwide mortality rate for leishmaniasis is between 26,000 and 65,000 cases per year (4). VL is revealed to be endemic in 7 areas in Iran and the most significant endemic foci are Ardabil Province in northwestern Iran (5). Untreated VL has a 100% mortality rate (6). Of 44 confirmed species of sand flies in Iran, three of them have been identified as the main or probable vectors of VL in Ardabil Province (7-10). To date, 22 *Phlebotomus* species have been recognized in Ardabil Province, and two major vector species, *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus*, have been recorded from various regions with a large population (11). Leishmania infection of the two listed species with *L. infantum* and *Leishmania donovani* from the north and center of the province has been reported using parasitological and molecular techniques (12). At present, the chemical control of sand flies, including insecticide residual spraying (IRS) or the use of insecticide-impregnated bed nets (ITNs), are effective means to control visceral leishmaniasis (13). In order to use insecticides against leishmaniasis, the WHO recommends the use of a safety insecticide previously tested for vectors' susceptibility (14-20). Accordingly, this is the first study to test the sensitivity of two major vectors of visceral leishmaniasis in an endemic foci in northwestern Iran to four types of insecticides based on the WHO guidelines.

Materials and Methods

Study sites

This research was performed in Ardabil Province located in northwestern Iran from May to October 2019. The geographic coordinates of the province are 38.4853 °N, 47.8911 °E. The province is 17,953km² in area (about 1.09 percent of the country's total region), 1,338 meters above sea level and has a population of 1,249,000 people.

Sandflies collection

A total of 1,278 sand flies were collected for testing and control purposes. Samples were gathered from the villages of Meshkinshahr and Germe Districts using light traps and aspirators from human homes, dog nests and livestock stables. Sand flies were immediately transferred to the Arthropod-Borne Research Center in Ardabil University of Medical Sciences. The specimens were kept in a laboratory in a maintenance cage under 25±2 °C and 60±10% humidity. The specimens were fed with sugar-soaked cotton during storage. They were recognized by using valid keys (21-23).

Procurement of insecticide impregnated papers and their concentration

Impregnated papers with DDT (4%) (Batch No. DD186, Exp. date: July 2016); Malathion (5%) (Batch No. MA 177, Exp. date: April 2018); Propoxur (0.1%) (Batch No. PR 104, Exp. date: April 2018), and Lambda-cyhalothrin (0.05%) (Batch No. LA 262, Exp. date: July 2018), as well as papers for control were supplied by the collaborating center of the World Health Organization in Penang, Malaysia.

Susceptibility tests

Female sandflies were subjected to susceptibility tests according to the guidelines of WHO. Sandflies were exposed to impregnated papers (DDT (4%) (Organochlorine), Malathion (5%) (Organophosphate), Propoxur (0.1%) (Carbamate) and Lambda-cyhalothrin (0.05%) (Pyrethroid) at different logarithmic times.

The sample exposure period was 3.75, 7.5, 15, 30 and 60 minutes. Samples were recovered at a suitable temperature for 24 hours under laboratory circumstances and supplied with sugar water. The mortality rate was recorded after 24 hours. Dead and live samples were gathered and stored in 70% alcohol and then mounted in a drop of Puri's medium and recognized using valid morphological keys. Four replicates were performed for each experiment.

Data analysis

Data collected in Microsoft Excel 2016 were used to plot slopes and graphs. The MedCalc statistical software was used to calculate LT_{50} and LT_{90} and to compare the chances of sand flies surviving the exposure to insecticides in the study areas.

Results

During the study period, 1,278 sand fly specimens were used for sensitivity test on two main proven vectors species of VL in the

Ardabil Province of which 1,063 were for the test and 215 for the control group. Seven hundred and forty-nine specimens of *Ph. kandelakii* and 529 specimens of *Ph. perfiliewi transcausicus* were evaluated respectively. All the samples of the above-mentioned species were found fully susceptible to Lambda-cyhalothrin (0.05%) insecticides with 100% mortality. The estimated LT_{50} values for DDT (4%), Malathion (5%), Propoxur (0.1%), and Lambda-cyhalothrin (0.05%) for *Ph. kandelakii* were 15.1, 13.4, 15.4 and 5.8 minutes (Table 1), respectively, and for *Ph. perfiliewi transcausicus* were 11.9, 15.6, 15.9 and 5.8 minutes respectively (Table 2).

The mortality rates for *Ph. kandelakii* after 60 minutes of exposure to DDT (4%), Malathion (5%), Propoxur (0.1%) and Lambda-cyhalothrin (0.05%) were 91.17%, 93.18%, 97.61% and 100%, respectively. The mortality rates for *Ph. perfiliewi transcausicus* were 95.83%, 90.90%, 91.30% and 100%, respectively (Table 3). The slope diagrams and the trend of the mortality rates are shown in Fig. 2.

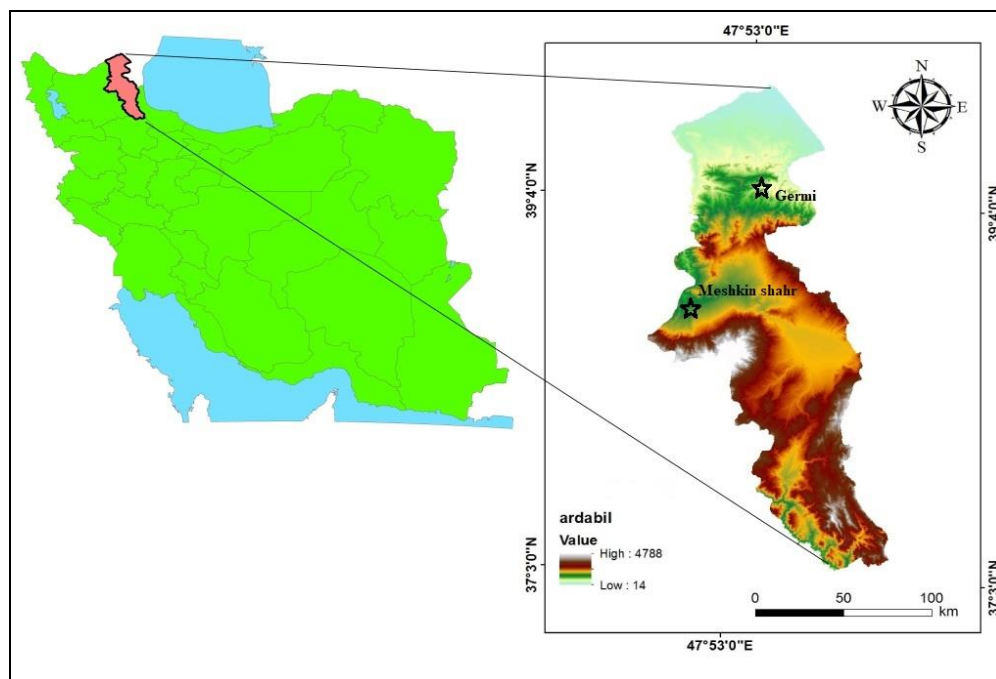


Fig. 1. The study area and sand fly collection sites in the northwest of Iran

Table 1. Lethal Time, LT₅₀ and LT₉₀ values for *Phlebotomus kandelakii* to DDT (4%), Malathion (5%), Lambda-cyhalothrin (0.05%) and Propoxur (0.1%), in Ardabil Province, Iran, 2019

Insecticide	a	b±SE	LT ₅₀ ±95% CI (minutes)	LT ₉₀ ±95% CI (minutes)	Wald statistic	p-value
DDT (4%)	-3.063	2.596±0.367	11.884	34.783	49.855	<0.0001
			15.123	47.113		
			18.943	76.134		
Malathion (5%)	-3.144	2.789±0.338	10.911	29.906	68.032	<0.0001
			13.397	38.582		
			16.235	56.021		
Lambda-cyhalothrin (0.05%)	-2.501	3.278±0.551	4.084	10.012	35.296	<0.0001
			5.794	14.253		
			8.221	20.292		
Propoxur (0.1%)	-3.060	2.579±0.326	12.375	36.518	62.416	<0.0001
			15.351	48.183		
			18.811	72.833		

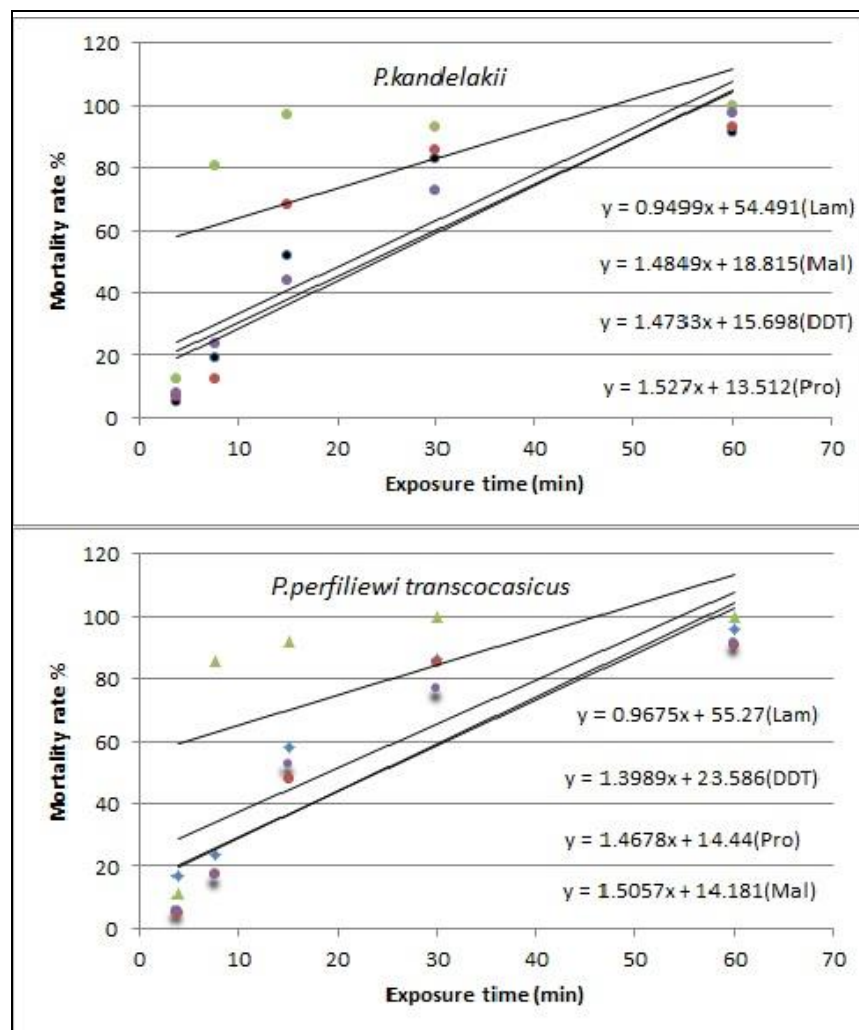


Fig. 2. Comparison of regression lines, equations and LT₅₀ of *Phlebotomus kandelakii* and *Ph. perfiliewi transcaspicus* as exposed to DDT (4%), Malathion (5%), Propoxur (0.1%), and Lambda-cyhalothrin (0.05%)

Table 2. Lethal Time , LT₅₀ and LT₉₀ values for *Phlebotomus perfiliewi transcaucasicus* to DDT (4%), Malathion (5%), Lambda-cyhalothrin (0.05%) and Propoxur (0.1%), in Ardabil Province, Iran, 2019

Insecticide	a	b±SE	LT ₅₀ ±95% CI (minutes)	LT ₉₀ ±95% CI (minutes)	Wald statistic	p-value
DDT (4%)	-2.621	2.436±0.402	8.703	27.897	36.682	<0.0001
			11.901	39.949		
			15.725	73.761		
Malathion (5%)	-3.207	2.690±0.431	11.757	32.870	38.864	<0.0001
			15.565	46.619		
			20.495	84.098		
Lambda-cyhalothrin (0.05%)	-3.430	4.506±0.908	4.467	8.821	24.606	<0.0001
			5.772	11.111		
			7.059	17.158		
Propoxur (0.1%)	-3.089	2.568±0.404	12.221	35.565	40.336	<0.0001
			15.944	50.291		
			20.679	90.236		

Table 3. Susceptibility tests using the WHO bioassays for *Phlebotomus kandelakii* and *Ph. perfiliewi transcaucasicus* in endemic foci of visceral leishmaniasis in the northwest of Iran, 2019

Insecticide	Exposer time (min)	<i>Ph. kandelakii</i>			<i>Ph. perfiliewi transcaucasicus</i>		
		No. of sandflies exposed	No. of sand- flies dead	Mortality rate (%)	No. of sand- flies exposed	No. of sand- flies dead	Mortality rate (%)
DDT (4%)	3.75	20	1	5	18	3	16.67
	7.5	26	5	19.23	21	5	23.80
	15	31	16	51.61	19	11	57.89
	30	29	24	82.75	22	19	86.36
	60	34	31	91.17	24	23	95.83
Malathion (5%)	3.75	29	2	6.89	21	1	4.76
	7.5	32	4	12.5	17	3	17.64
	15	38	26	68.42	19	9	47.36
	30	35	30	85.71	20	17	85
	60	44	41	93.18	22	20	90.90
Lambda-cyhalothrin (0.05%)	3.75	24	3	12.5	18	2	11.11
	7.5	26	21	80.76	21	18	85.71
	15	31	30	96.77	25	23	92
	30	28	26	92.85	19	19	100
	60	38	38	100	26	26	100
Propoxur (0.1%)	3.75	26	2	7.69	18	1	5.56
	7.5	34	8	23.52	24	4	16.67
	15	32	14	43.75	21	11	52.38
	30	40	29	72.50	26	20	76.92
	60	42	41	97.61	23	21	91.30
Control	-	110	2	1.81	105	3	2.85

Discussion

Since Ardabil Province, along with East Azerbaijan, is known as the primary focal point of VL in Iran (24) and 100–300 new cases of

the disease are recorded annually (5), most of which are from the northwest (25), care must be taken against any sudden epidemics. One

way to control the epidemic is to use suitable and safe insecticides to kill sand flies (22). Several trials have been performed in separate areas of Iran on vectors of cutaneous leishmaniasis, including *Ph. papatasi* and *Ph. sergenti*, and it has been verified that *Ph. papatasi* is susceptible to the assessed insecticides (26–29), except tolerance to DDT in Isfahan Province, central Iran (30).

Furthermore for the first time, resistance against DDT was observed in wild strain of *P. sergenti* population in North Khorasan Province, Iran (14, 27). According to the results of current study, Lambda-cyhalothrin 0.05% with 100% lethality had the greatest impact on *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus* in Ardabil region. This revealed that these vectors are extremely susceptible to this insecticide and can be used to regulate vectors at the period of the epidemic. The results of the Lambda-cyhalothrin (0.05%) insecticide in Brazil against *lutzomyia longipalpis*, the main vector of VL in the Americas, showed that this vector was also highly sensitive to insecticides (31).

The *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus* vectors' resistance to insecticides has not been recorded to date in Iran. In 1994, susceptibility tests were conducted on *Ph. kandelakii* and *P. perfiliewi transcaucasicus*, in Ardabil Province (northwest), Iran. The results revealed that the both species were susceptible to the insecticides and mortality rate with 60min exposure to 4% DDT was 100%. In India, in numerous areas, *Ph. argentipes* showed resistance to DDT (32–34), but was susceptible to malathion and deltamethrin with 98% and 100% mortality, respectively (20). The resistance of the main vectors of VL against DDT (4%) and deltamethrin (0.05%) was reported in borderline of Nepal (17).

The reports on insecticide susceptibility status of *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus* are limited and there are no records of insecticide resistance till now. According to results of current study and the WHO guide-

lines for malaria vectors at the diagnostic dose *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus*, proven vectors of visceral leishmaniasis in northwestern Iran were possible resistance to Propoxur (0.1%), Malathion (5%) and DDT (4%) insecticides. So the results of earlier studies were contradictory to this study. *Phlebotomus kandelakii* distributed in some important VL foci of Iran and *leishmania* infection reported previously so further studies should be conducted in other regions (8).

The strength of this study was that we conducted insecticide susceptibility status of two main vectors of VL in an endemic area of Iran with four different kinds of insecticides and in five different logarithmic times but because of limitation, the enough amount of sand flies collection was not possible. Also there are specific guidelines approved by WHO for diagnostic dose of mosquitoes, and there are no such guidelines for phlebotominae sandflies; however, it is of great value to establish a baseline data and to assess the insecticide susceptibility to different insecticides in sand fly vectors. There are several novel investigations of main vector borne diseases in the country (35–38). Evaluation of diseases monitoring is a vital responsibility of corresponding ministries.

Conclusion

The findings of this study showed that according to the criteria for resistant level of *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus*, the proven vectors of VL in northwestern Iran were susceptible to Lambda-cyhalothrin (0.05%), however these species are possible resistance to Propoxur (0.1%), Malathion (5%) and DDT (4%) insecticides. Regular monitoring for the resistance of *Ph. kandelakii* and *Ph. perfiliewi transcaucasicus* sand fly populations are necessary for VL control programs in this area. Furthermore, these insecticides should also be assessed for *Ph. papatasi* and *Ph. sergenti*, as the main vectors of CL in endemic foci in Iran.

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