Original Article

Comparative Testing of Susceptibility Levels of Phlebotomus sergenti, the Main Vector of Anthroponotic Cutaneous Leishmaniasis, to Conventional Insecticides Using Two Capture Methods in Kerman City, Southeastern Iran

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Abstract

Background: Collecting live sand flies from indoor sites is a major challenge for researchers in large cities due to the reluctance of families to survey their homes. This study was conducted to assess the efficacy of two methods for collecting sand flies for use in susceptibility tests in the urban area of Kerman, southeastern Iran.

Methods: Sandflies were mainly collected using both baited traps and hand catch methods from outdoor and indoor sites. Susceptibility tests were separately done according to the standard World Health Organization testing protocol on *Phlebotomus sergenti*, including 60-minute exposure to DDT 4.0%, propoxur 0.1%, deltamethrin 0.05%, and malathion 5.0%.

Results: During this research, the natural habitats and suitable indoor sites were selected to predict the density of live sand fly with perfect accuracy. The number of live *Ph. sergenti* caught by hand catch and baited traps methods was 42 and 361 in indoor and outdoor sites, respectively. The mortality rate of *Ph. sergenti* exposed to DDT 4%, deltamethrin 0.05, malathion 5%, and propoxur was 100%.

Conclusion*:* The baited traps showed a significant efficiency compared to hand catch for collecting live *Ph. sergenti* for use in susceptibility tests in urban areas. The *Ph. sergenti* collected from both indoor and outdoor sites were susceptible to all insecticides.

Keywords: *Phlebotomus sergenti*; Susceptibility; Insecticides; Baited traps; Hand catch

Introduction

Anthroponotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica* is transmitted by *Phlebotomus sergenti* (1). In the ACL foci, the humans could act as a *L. tropica* reservoir for sand fly infection during the year. In this regard, patients with acute ulcers (2), chronic diffuse ulcers, lupoid or tuberculous ulcers (3), non-healing ulcers (4), recurrent ulcers (5) and ulcers resistant to pentavalent antimonial drugs could provide enough parasitic reservoirs for establishing an ACL transmission cycle by sandflies in the area (6). Dogs are considered as an accidental host (7) in which the lesions usually appear on the snout, interdigit, and sometimes on the corner of the eyelids (1, 8-9). The majority of ACL cases in the world occur in Morocco (10-11), Algeria (12), Libya (13), Tunisia (14-15), Afghanistan (16-21), Iran (22), Pakistan (23), Saudi Arabia (24), Syria (25), Jordan (26-27), Iraq (27), Israel (28), and Turkey (29). Anthroponotic cutaneous leishmaniasis is a well-known disease in densely popu-

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lated cities in Iran and is predominantly endemic in urban and peri-urban areas including Tehran (30), Mashhad (31), Neishabour (32), Shiraz (33), Kerman (34-35), Bam (37-40), Isfahan (41), and Jiroft (42). *Phlebotomus sergenti* is a proven vector of ACL (43). Dissection of many specimens of this species in Tehran failed to show the *Leishmania* (1), while there are several reports of the leptomonad of *L. tropica* in dissected *Ph. sergenti* in Mashhad (44). Moreover, *Leishmania* parasites have been found in Kerman (45-46) and Shiraz using restriction fragment length polymorphism (RFLP) (47-49). Leptomonad infections of *Ph. sergenti* have also been reported from Afghanistan, Saudi Arabia (50) and Tunisia (51). In some urban foci, *Ph. caucasicus* is suspected as a secondary vector (1). In the ACL foci located in the plains, the behavior of *Ph. sergenti* is completely domestic with more density found at indoor sites versus the mountainous ACL foci. This species is found in moderate density in caves and crevices of mountains and foothills where the birds act as a host for blood feeding. The presence of light or other avian hosts attracts the sandflies to the houses located adjacent to the foothills or mountains (43). In southern ACL foci, the activity of *Ph. sergenti* begins in late June and ends in mid-September (52). In the central ACL foci, the activity starts in early April and ends in mid-November with two peaks at the end of April and in mid-September (53). Some ecological aspects and surveillance of *Ph. sergenti* have been studied in endemic foci of ACL (54-57). The ACL occurrence and emergence are characterized by several factors such as environmental changes, poor housing conditions, unprotected people from sand fly bites, resettlement of non-immune people, deterioration of the infrastructure and migration (58-62), natural disasters like the earthquake (63-65), displacement of people in borderlines (66), civil wars (67-69), and refugee camps (70-76). At present, spraying operations are limited to the residual foci of malaria in the country (79) and this method is not applied for controlling the ACL vectors. Based on the endophilic habits of *Ph. sergenti*, some attempts have been made to reduce the density of sandflies (78-81) using other insecticide-based vector control measures such as insecticide impregnation of the bed nets (ITNs) (82-86), curtains (ITCs) (87-88), tents (ITTs) (89). Moreover, integrated vector control methods (89-90) have also been applied to control *Ph. sergenti* population in epidemic conditions. In order to analyze the susceptibility tests on sand flies, testing of insecticides at discriminative doses has been used for ranking the susceptibility level and as a criterion for interpreting the results. It is difficult to collect live sandflies from indoor places in large cities. Therefore, the present study was conducted to determine the most convenient capture method for collecting live sandflies from indoor and outdoor sites and to use the collected sand flies for determining their susceptibility to conventional insecticides in the ACL foci in Kerman, southeastern Iran, where there is scarce data in this regard.

Materials and Methods

Study area

The study area was located in Kerman, southeastern Iran. The city borders Khorasan and Yazd to the north, the Lut Desert to the east, Bam to the south, Sirjan to the west, and Rafsanjan to the northwest. Kerman is elevated 1793 meters above the sea level and the main coordinates of the study locations were between 30°06'32"N and 57°06'27.6"E. The study area was a wide plain surrounded by stony mountains with a poor vegetation. The studied localities for collecting the adult sand flies were Sarasiyab, Masjed-e-Sahebzaman, Shahzadeh-Mohammad, Safa Cave, Allahabad, Shahrak-Sanaati, Sarbaz Mountains and Shahrak-e-Almahdi (Fig. 1). The entomological operations were carried out from July to September 2019.

Sand fly collection

Live sand flies were collected using an as-

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pirator from the homes located in the periphery of the mountains in the early morning. The collected sand flies were released into paper cups and transferred to the laboratory for testing. The baited traps were equipped with mini gas lamps, and the netted tents were installed close to the caves or crevices near the mountains around Kerman City (Fig. 2), where the presence of adult sand flies was already proven during pretest trials using sticky paper traps. The collected live sand flies were also released into paper cups and transferred in a box under a wet towel. Sucrose solution was provided on the top of cups as a cotton pad soaked in sucrose solution 10%.

Tested insecticides

The insecticide-impregnated papers were purchased from the WHO Representative in Penang, Malaysia. The papers were impregnated with DDT 4.0% (batch number DD 265, expire date: July 2022), deltamethrin 0.05% (batch number DE 527, expire date: August 2019), malathion 5.0% (batch number MA 234, expire date: July 2020), and propoxur 0.1%, (batch number PR, 123 expire date: August 2020) and tested using the WHO's testing kit.

Susceptibility tests

Susceptibility tests were carried out on sand flies collected from both indoor and outdoor sites. The collected sandflies were tested separately according to the capture method using the World Health Organization (WHO) protocols (91-93). The mortality rates were determined at discriminative doses for 60 minutes using the WHO test tubes for the adult sand flies. At each replicate, 20–25 sand flies were tested. A 10% sucrose solution was provided during the recovery period. The mortality was recorded after 24 hours of the recovery period. Both dead and live sandflies were preserved in separate tubes containing 70% ethanol according to tested insecticides and collecting methods until microscopic slides were prepared using the Puri's medium at the appropriate time. The mounted sandflies were identified using valid identification keys (94-96).

Data Analysis

The susceptibility data including the number of live, dead, and total sandflies were ranked according to sex (male and female) and physiological condition (blood-fed, unfed and gravid) separately in two treatment groups compared to the control group. The charts of mortality rate were drawn with standard errors to show any significant differences using the Microsoft Excel 2010 software. The independent t-test was applied to compare the abundance and sex ratio of *Ph. sergenti* between hand-catch and baited trap methods using the IBM SPSS software version (25).

Results

Efficiency of collection methods

The efficiency the baited traps for collecting live *Ph. sergenti* from outdoor was 370 compared to 45 sand flies collected using hand-catch from indoor sites (Table 1) indicating an significant difference ($t= 7.214$, $df= 9$, $p= 0.001$) (Fig. 3). The sex ratio of *Ph. sergenti* was 1.0 at indoor compared to 1.2 at outdoor places (Table 1) showing no significance difference ($t= 2.574$, $df= 8$, $p= 0.07$) (Fig. 4).

Susceptibility of *Phlebotomus sergenti* collected from indoors

The mortality rate of *Ph. sergenti* collected from indoor places using hand-catch method was 100% after exposure to DDT and malathion. The mortality rate was 0.0% in the control group (Fig. 5).

Susceptibility of *Phlebotomus sergenti* collected from outdoors

The mortality rate of *Ph. sergenti* collected from outdoor sites using baited traps was 100 %. After exposed to DDT, propoxur, deltamethrin, and malathion. The mortality rate was 0.0% in the control group (Fig. 6).

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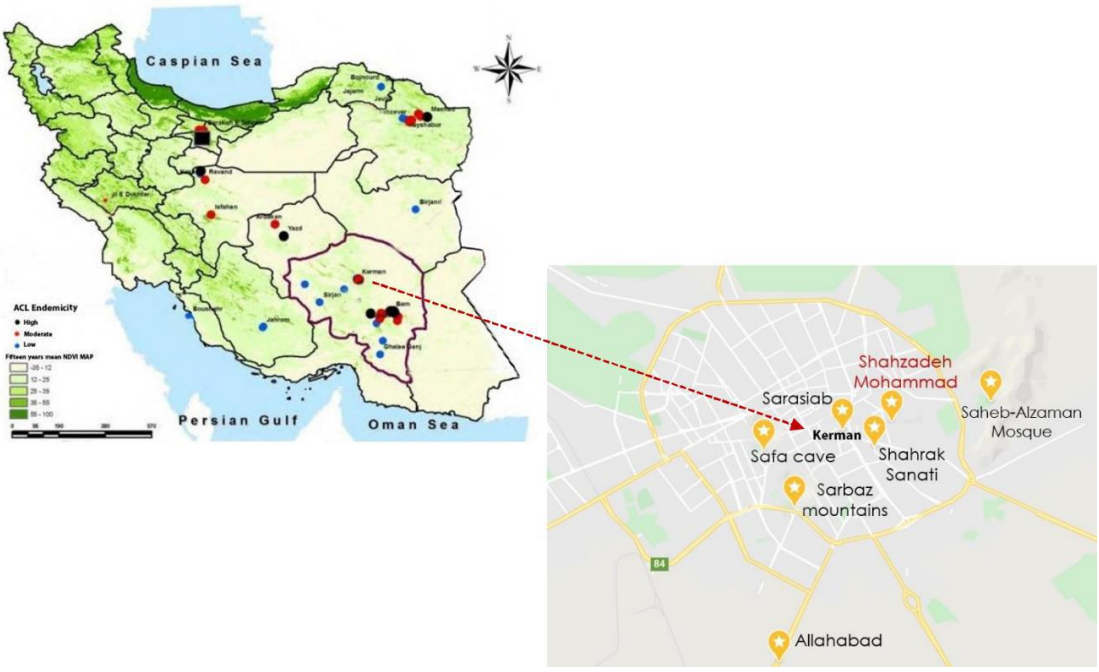

Fig. 1. Map showing Kerman City and the locations studied the collecting methods of live sand flies used for susceptibility tests, Kerman Province, 2019

Fig. 2. Using the baited traps for collection of live *Phlebotomus sergenti* in the mountainous areas around Kerman City, southeastern Iran, Kerman Province, 2019

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Table 1. Statistical comparison of efficiency of two collection methods used for live collection of *Phlebotomus sergenti*, Kerman City, southeastern Iran, July–Sep 2019

Capture methods	Capture place	Sessions of collection	Total collected	Capture Rate (%)	No. male	No. female	Sex ratio	Physiologic conditions			Environmental condition	
								No. fed	No. unfed	No. gravid	Temp °C	Relative humidity (%)
Baited traps	Outdoor	9	370	89.2*	198	172	1.2†	29 (16.8%)	112 (65.1%)	31 (18.0%)	28–30	<40
Hand-catch	Indoor	5	45	10.8*	22	23	1.0†	3 (17.4%)	17 (73.9%)	2 (8.7%)	26–28	<40

T=-7.214, df= 9, p= 0.001
† t=-2.574, df= 8, p= 0.07

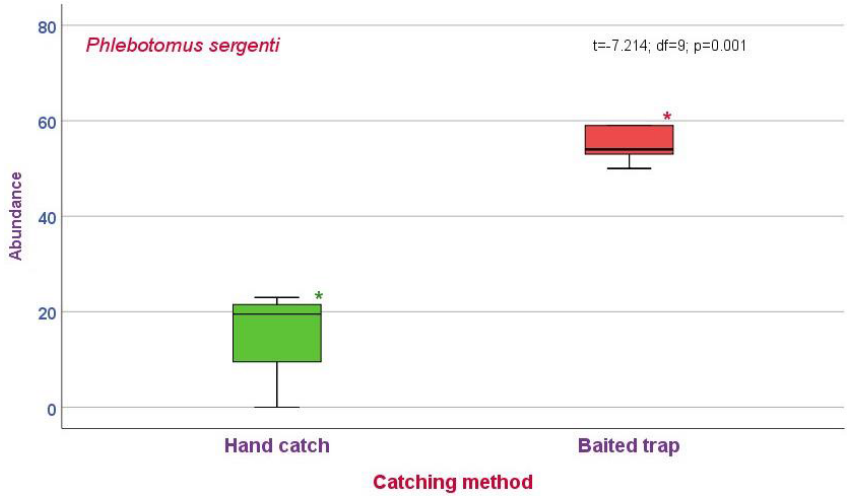

Fig. 3. Statistical comparison of abundance of *Phlebotomus sergenti* collected using hand-catch and baited traps from indoor and outdoor places, Kerman City, southeastern Iran, 2019

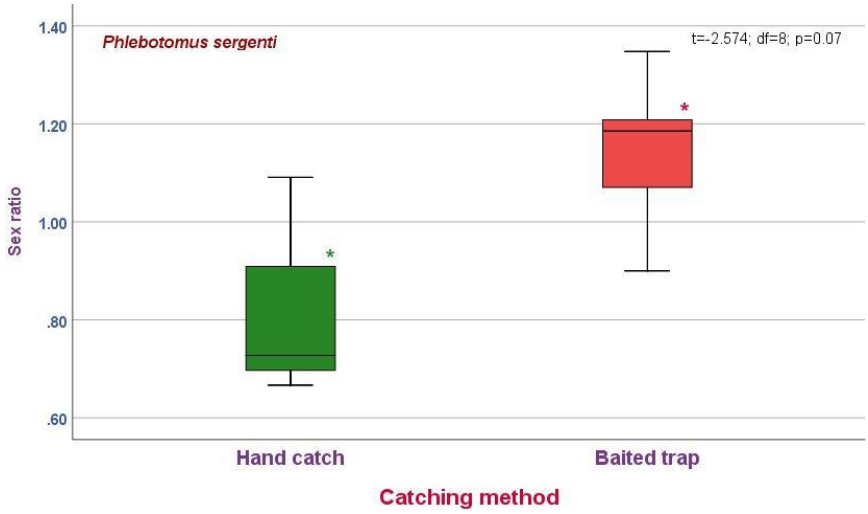

Fig. 4. Statistical comparison of sex ratio of Phlebotomus sergenti collected using hand-catch and baited traps from indoor and outdoor places, Kerman City, southeastern Iran, 2019

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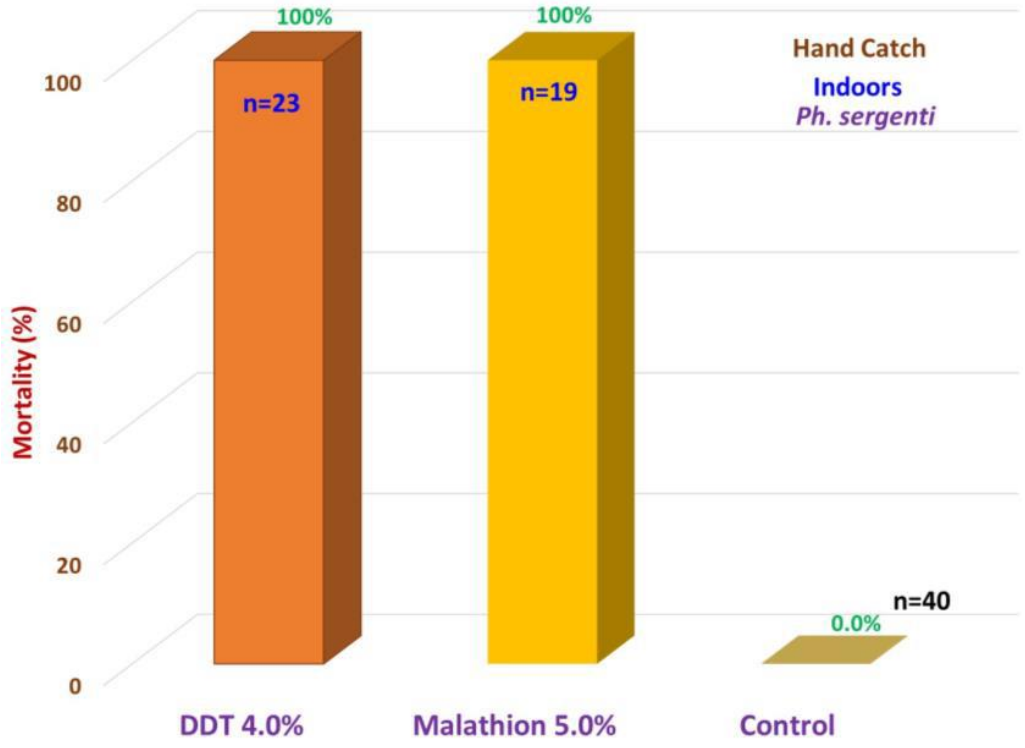

Fig. 5. Susceptibility of *Phlebotomus sergenti* to DDT and malathion, collected using hand-catch from indoor places, Kerman City, southeastern Iran, 2019

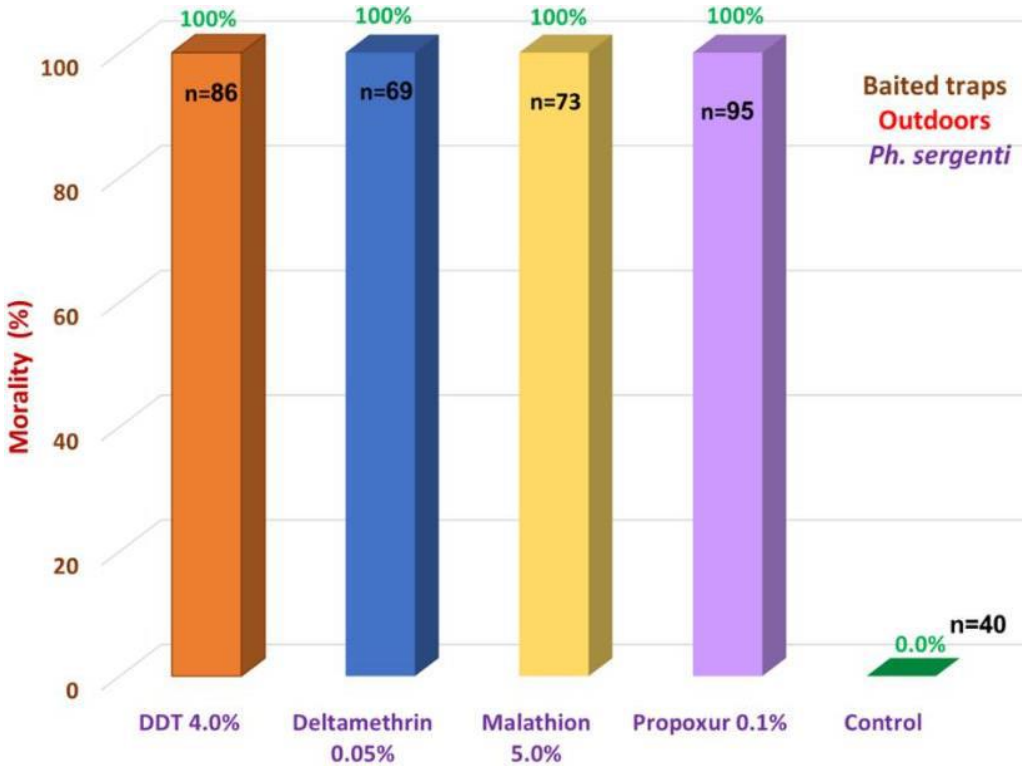

Fig. 6. Susceptibility of *Phlebotomus sergenti* to DDT, deltamethrin, malathion and propoxur collected using baited traps from outdoor places, Kerman City, southeastern Iran, 2019

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Discussion

Despite the implementation of the national leishmaniasis surveillance program and the efforts for adopting different control methods during past decades, ACL is still a major health challenge with increasing trends in many parts of Iran, particularly Kerman Province. This study was conducted to investigate the susceptibility level of *Ph. sergenti* to conventional insecticides according to the WHO instruction for the phlebotomine susceptibility test. Furthermore, there is an operational gap for employing a capture method for adequate yielding of live *Ph. sergenti*, which is necessary for the standard sample sizes used in susceptibility tests. In this study, efficiency of baited traps showed the more yield of live *Ph. sergenti* compared to hand-catch method from indoor places (t= 7.214, df= 9, p= 0.001) with no significant difference for the sex ratio. The most important obstacle for collecting sand flies from indoor places using the hand-catch method is the lack of cooperation and willingness of the households to check their houses for the presence of sand flies. In addition, in urban areas, due to the application of aerosol sprays or using cooling systems in the houses, the abundance of sand flies is usually lower than the suburbs. In the other study, different collecting methods were assessed for live sand flies with a total of 122 live *Ph. sergenti* collected at outdoor sites in the mountainous parts of Kashan District. The comparison of different traps showed the most efficiency (59.8%) for the black Shannon traps during the summer (97). In other attempts made in the northeastern parts of Iran as well as in Cukurova Plain, southern Anatolia, Turkey, the CO2 CDC light traps showed the most efficiency with 43.2% and 75.0 percent for collecting live *Ph. sergenti* with a total abundance of 2521 and 4 from outdoor sites (98-99). In another testing, different commercial traps were assessed for collecting live *Ph. sergenti* in the north of Aswan, southern Egypt which the BGS traps showed more efficacy (35.5%) (100). The results of susceptibility tests on *Ph. sergenti* collected from outdoor sites showed 100% mortality exposed to DDT 4.0%, deltamethrin 0.05%, malathion 5.0%, and propoxur 0.1%, indicating the complete susceptibility. The *Ph. sergenti* caught in the houses located adjacent to the mountains using hand-catch method revealed 100% mortality when exposed to DDT 4.0% and malathion 5.0%. Due to the obvious differences in the biology and behavior of *Ph. sergenti* with other species of sand flies, the finding of the present study was compared with other studies if the tests were only carried out on *Ph. sergenti*. The number of reports on the susceptibility level of *Ph. sergenti* to insecticides is limited in the world. During the years 1971, 1976, and 1998, three trials were carried out in the ACL foci in the large cities located in the northeast including Mashhad (31) and Neishabur (32) as well as Kerman (101) located in the southeast of Iran. The findings revealed the complete susceptibility of this species to the DDT. Moreover, there are three other published records for assessing the susceptibility status of *Ph. sergenti* in Mashhad, northeast of Iran (102) and Dehbakri County, southeast of Iran (103) in 2007 and 2011, which indicated the complete susceptibility of *Ph. sergenti* to DDT 4.0% and deltamethrin 0.05%. In a more recent study carried out in Maneh and Samalqan County, northeastern Iran, all sand flies were collected using CDC light traps from outdoor and tested with papers impregnated with DDT 4.0%, bendiocarb 0.1%, and permethrin 0.75%. The results showed mortality rates of 89.8±1.4, 93.6±1.4, and 95.6±1.7% respectively, indicating the resistance of *Ph. sergenti* to DDT (104). A study carried out in North Africa and the Middle East in 2007 found the high susceptibility of *Ph. sergenti* to malathion, DDT, cyfluthrin, bendiocarb, permethrin, and resmethrin (105). A susceptibility test conducted in Morocco showed the full susceptibility of wild populations of *Ph. sergenti* to DDT, lambdacyhalothrin, and malathion (106). The finding of the present research showed the susceptibility of *Ph. sergenti*

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to DDT, malathion, deltamethrin, and propoxur in indoor and outdoor populations collected in the ACL foci of Kerman. It is expected that application of the insecticide-based control strategies for sand flies, for example IRS, ITNs, ITC and ITT, could be an effective method for controlling *Ph. sergenti* in indoor and outdoor places if the measures are performed correctly.

Conclusion

Anthroponotic cutaneous leishmaniasis is still a major health problem in Kerman, southeast of Iran. This research showed the high susceptible status of *Ph. sergenti* in both the indoor and outdoor populations against all tested insecticides. The research-based evidence indicates the success of future insecticide-based vector control methods for controlling sand flies in the ACL foci.

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