Original Article

Efficiency of Two Capture Methods Providing Live Sand Flies and Assessment the Susceptibility Status of *Phlebotomus papatasi* (Diptera: Psychodidae) in the Foci of Cutaneous Leishmaniasis, Lorestan Province, Western Iran

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Abstract

Background: The aims of this study were to evaluate the efficiency of two capture methods for providing live sandflies used for determining the susceptibility level of *Phlebotomus papatasi*, the main vector of zoonotic cutaneous leishmaniasis in Lorestan Province, west of Iran.

Methods: The sand flies were collected from indoor and outdoor by hand-catch and baited traps during the peak of seasonal activity. The susceptibility level of sand flies was assessed using insecticide-impregnated papers against DDT 4%, bendiocarb 0.1%, permethrin 0.75%, deltamethrin 0.05%, and cyfluthrin 0.15%.

Results: A total of 2486 live sandflies were caught from both indoor and outdoor places. Totally 849 sand flies were caught from outdoors with a sex ratio(SR) 0.1 versus 1637 sand flies collected from indoor using the hand-catch method with SR= 0.6. The dominant species of sand flies was *Ph. papatasi* in the study area. Mortality rates of outdoor-collected sand flies were exposed to DDT 4%, deltamethrin 0.05%, permethrin 0.75%, and bendiocarb 0.1%, and mortality rate ranged from 92.0–97.9% and for indoor-collected sand flies were 87.7–96.8%. Both outdoor and indoor collected sand flies were susceptible to cyfluthrin 0.15% that caused 100% mortality.

Conclusion: Based on the findings, the most appropriate method for collecting the live female *Ph. papatasi* is the baited traps due to providing enough females is necessary for conducting the susceptibility tests. The finding indicated that *Ph. papatasi* was resistant to DDT, under 'verification required' status to deltamethrin, permethrin, bendiocarb, and susceptible to cyfluthrin.

Keywords: *Phlebotomus papatasi*; Insecticides; Resistance; Baited traps; Hand-catch

Introduction

Phlebotomus papatasi was the main vector of zoonotic cutaneous leishmaniasis (ZCL) and papatasi fever in the past. This species is prevalent in many areas in thewestern Palaearctic Region including Europe (France, Spain, Greece, Poland, Czech Republic, and Italy), Russia, and Central Asia as well as all countries in the Eastern Mediterranean Region, the western and northern Ethiopian Region (Saudi Arabia, Yemen, Ethiopia, and Sudan) and the Oriental Region (India and Bangladesh). *Phlebotomus papatasi* is found almost in all provinces of Iran (1). It prefers the climatical conditions with hot summers, temperate winters, and monsoon rains and the minimum temperature should not less than -6 ˚C. This species prefers the environmental moisture and warmth and is frequently caught in areas where the water has a high level (2). Numerous biological observations on *Ph. papatasi* was indicative of the higher abundance in the plains compared to high mountainous regions. It has been recurrently

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found in rodent burrows, human and domestic animal shelters in the central plateau of Iran (3). *Phlebotomus papatasi* is more prevalent at indoor compared to other species of sand flies. The larval stage develops at animal shelters and rodent burrows (4). In terms of endophilic behavior, *Ph. papatasi* generally rests in human and animal shelters and becomes as a domestic species in many regions, so that no sand fly could ever be so accustomed to adapting to the residential places (5). With development the construction of human dwelling in the plains, abundance of *Ph. papatasi* rapidly increases and when the settlements are near to ZCL foci, the transmission condition is more likely to become epidemic (6). It seems that the sandflies, which were caught from residential places, moved from adjacent infected rodent burrows, where *Ph. papatasi* is considered as the prevailing species (7). In terms of biting and blood-feeding behaviors, *Ph. papatasi* can repeatedly feed on its hosts both indoor and outdoor. The presence of multiple lesions on affected persons indicating evidence for recurrent bites of humans by sand flies (8). Considering the blood-feeding preference of *Ph. papatasi*, it can be argued that it mainly feeds on any host available nearby with more tendency to humans and rodents. The seasonal activity of this species starts from the beginning of May to the end of October in the northern and central regions of the country and from mid-April to January in southern regions with peaks in July and September (10). The lepomonad infection of *Ph. papatasi* has been reported from Isfahan, Lotfabad, Esferayen, Torkaman Sahra, Ahvaz, Dezful, Shush, Abardej, Balochistan, Sarakhs, Semnan, Damghan and Shahrud in Iran, all of which are known as the foci of ZCL (11-13). Transmission of ZCL by *Ph. papatasi* occurs outside the residential places, especially on the rooftops of houses or outdoor in front of the house in the regions with high endemicity for ZCL, whilst in regions with low endemicity, the transmission is more common in agricultural fields and in localities near the rodent burrows (10). Therefore, the transmission chain of ZCL seems to be interrupted by the use of pyrethroids impregnated bed nets, curtains, or uniforms and personal protection using repellents and occasionally application of residual spraying at indoors (14). The preliminary trials for determining the susceptibility levels of *Ph. papatasi* were reported in the Jordan Valley in 1967 (15) and the subsequent report revealed the resistance in northern Bihar, India (16) and followed in Turkey (17). Although numerous susceptibility tests have been carried out in the foci of ZCL in Iran, no evidence has yet been presented for the resistance of *Ph. papatasi* to insecticides (18-22). This study was the first comparative trial to determine the susceptibility of *Ph. papatasi* collected from both indoor and outdoor in ZCL foci in Lorestan Province, west of Iran.

Materials and Methods

Study area

This study was conducted in Pol-e Dokhtar, Rumeshgan, and Kuhdasht districts where the highest prevalence of ZCL was reported. The geographical coordinates of the research sites were 47º27´–48º22´E, 32º37´–33º20´ N, the mean elevation 662 meters above sea level (AMSL) for Pol-e Dokhtar, 47º20´–48º65´E, 33º16´–33º35´N, 1089m AMSL for Rumeshgan, and 46º51´–47º50´E, 33º09´–33º56´N, and 1191m AMSL for Kuhdasht.

Sand fly collections

Sand flies were caught from indoor using hand catch method with mouth aspirators in the early morning as well as from outdoor using baited traps in the evening till midnight. Each baited double net trap had equipped with mini gas lamps as a light attraction as well as a rooster in a cage as an attractant host for blood-feeding of sand flies. The baited traps set up in the evening using four wooden bases, so that the lower edges of the nets lie 10

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cm above the ground. The location of traps was close to the rodent burrows and fox nests adjacent to the agricultural lands in the studied villages. The attracted sand flies were collected with the mouth aspirators also released into paper cups with net cover and transferred in a cool-box to the laboratory. A cotton pad soaked in 10% sucrose solution was put on the top of cups for feeding of sand flies.

Susceptibility tests

The insecticide-impregnated papers were purchased from the WHO's Collaborating Center, Malaysia. Susceptibility tests were carried out on both indoor and outdoor collected sand flies and exposed to DDT 4%, deltamethrin 0.05%, permethrin 0.75%, bendiocarb 0.1%, and cyfluthrin 0.15% impregnated papers using WHO test kits. At each replicate, 20–25 sand flies were exposed for 60min. If the mortality criterion was in the resistance border, the tests were continued using an increasing trend of logarithmic times until observing 98–100% mortality. Both living and dead sand flies treated with each insecticide were preserved in ethanol 70% till mounted in Puri's medium for species identification.

Data Analysis

Data of susceptibility tests including the number of live, dead, and total sand flies were grouped based on physiological conditions for each of the treated and control groups. The sex ratio is calculated by dividing the number of males by females. Mortality was calculated by counting the dead and live mosquito species after 24h exposure to insecticide paper. The mortality was then corrected by applying Abbott's formula when control mortality was observed between 5% and 20%, whereas tests with more than 20% control mortality were discarded and repeated. Median and mean of mortality used for plotting box and whisker plot used for showing the efficiency of two capture methods providing live sand flies and the independent-samples t-test used for showing a significant difference between two capture methods. WHO criteria were used for interpretation, with 98–100% mortality indicating susceptibility, 90–97% indicating needs further confirmation or verification required, and mortality < 90% indicating resistance. The Probit analysis was used in analyzing the time-mortality response to estimate LC_{50} for a fixed concentration of insecticides to kill a defined proportion of sand flies, known as lethal time (LT).

Result

Efficacy of two collecting methods

A total of 2486 live sandflies were caught using hand-catch and baited trap methods both from indoor and outdoor places with spending 12 and 4 working rounds. From indoor, 1637 sand flies (65.4%) were collected with sex ratio (SR) 0.6vs from outdoors 859 sand flies (34.6%) with SR= 0.1 (Table 1). A significant difference was shown between the SR values of sand flies collected in the hand-catch and baited trap methods (t= 7.245, df= 3, p= 0.005) (Fig. 1).

Susceptibility levels of *Phlebotomus papatasi* collected indoor and outdoor

The susceptibility of *Ph. papatasi* collected from both indoor and outdoor studied areas were assessd at discriminative doses and 60 min exposure time. The comparative percent mortality were 87.7±1.1 and 92.0±0.4 for DDT, 96.8±0.3 and 97.8±1.4 for deltamethrin, 92.4±0.9 and 97.9±0.0 for permethrin, 93.6± 0.9 and 94.0±0.7 for bendiocarb, and 100±0.0 and 100±0.0 for cyfluthrin while the values in the control group were 1.1±0.1% and 1.3±0.1 respectively (Fig. 2).

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Table 1. Efficacy of two capture methods used for collectiong live sand flies for susceptibility tests both indoors and outdoors, Lorestan Province, western Iran

Capture method	Type of collection place	Repetition of trial	Total live collected	Percentage	Male	Female	Sex ratio	Mean of Temperature (°C)	Relative humidity (%)
Baited trap	Outdoor	4	849	34.6	83	766	0.1	30	47
Hand-catch	Indoor	12	1637	65.4	1042	595	0.6	32	38

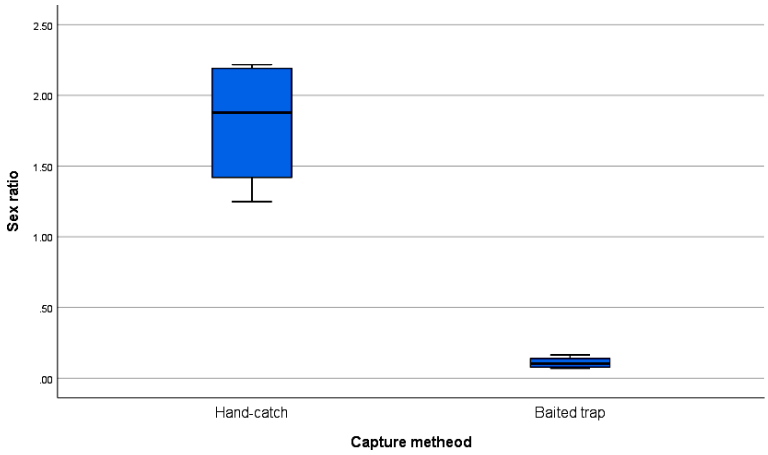

Fig. 1. Box plot showing sex ratio of sand flies abundance collected using hand-catch and baited traps methods, Lorestan Province, Iran

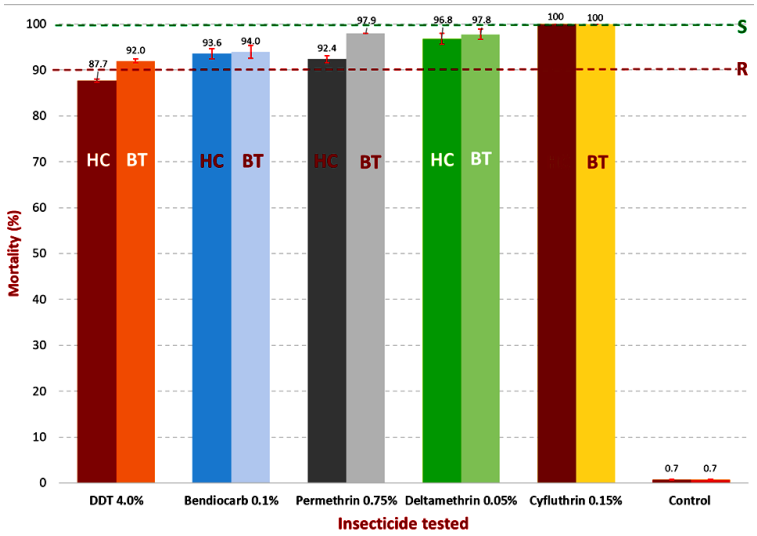

Fig. 2. Comparison of susceptibility level of *Phlebotomus papatasi* collected from indoor and outdoor with hand-catch and baited trap methods following the WHO's Criteria (32), Lorestan Province, Iran

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Discussion

Due to the widespread outbreaks of ZCL in Iran and the world, the practical approaches are interrupting the transmission chain using different individual protection methods such as insecticide-impregnated bed nets and curtains, application of repellent when working outdoors, and in limited scale application of indoor residual spraying (14). Prior to any control measures during outbreaks of ZCL, certain information is required about the bionomy of the vectors in order to take appropriate preventive measures during natural disasters and epidemics. Insecticides play a crucial role in controlling the vectors of ZCL, and the type of insecticide should be determined using standard tests before use (23). The larval habitats of sand flies are located in rodent burrows and other small mammals nests as well as in domestic animal shelters (24). Due to the extensive use of pesticides in animal husbandry and agricultural lands, the larval and adult sand flies are exposed to different chemicals which may lead to tolerant against various insecticides in the long term (25). Given that no comparative study has been conducted as a concurrent for determination on susceptibility level between two separate populations living indoors and outdoors using two live capture methods; this study was the first research attempt in this subject. According to the results of this study, the mortality of indoor-collected *Ph. papatasi* was 87%±1.1 when exposed 60min to DDT, and the for deltamethrin, permethrin, and bendiocarb were ranged from 92.4 to 96.8%. With regard to the sufficiency of test replication in this study, it became evident that *Ph. papatasi* was resistant to DDT, under'verification required' to bendiocarb, permethrin, and deltamethrin, and susceptible to cyfluthrin. The main cause for the occurrence of resistance to DDT is believed to be the frequent use of this insecticide for controlling malaria vectors during past decades, and the gene responsible for organochlorine resistance was transferred to the next generations (26). It has been reported that the increased use of pyrethroid insecticide the health and agriculture sectors has resulted in cross-resistance to both organochlorine and pyrethroid insecticides (27). The sand flies collected from outdoor using baited traps were tolerant to DDT 4%, deltamethrin 0.05%, permethrin 0.75%, and bendiocarb 0.1% and the mortality rates ranged from 92.0 to 97.8% comparison of susceptibility of *Ph. papatasi* collected from both indoor and outdoor using hand-catch baited traps methods showed the under 'verification required'/susceptibility of sand flies caught from outdoor compared to indoor against DDT, deltamethrin, permethrin, and bendiocarb. Investigations on the susceptibility of sand flies to insecticides were initiated by Seyedi Rashti in Mashhad, northeastern Iran in 1970. He found that *Ph. papatasi* was susceptible to DDT and dieldrin (18). A study in Isfahan showed no resistance in *Ph. papatasi* (12). According to research in Mashhad and Isfahan (2006–2007), the LT_{50} of *Ph. papatasi* was increased up to 2.3–3% (22). The first occurrence of under 'verification required' status to DDT was reported from Isfahan in 1992 (29), while another study in the Varzaneh County (Isfahan Province) showed complete susceptibility to DDT in the same period. In the latter study, it was concluded that the discontinuation application of the organochlorine insecticides could result in returning the under 'verification required' status (12). Some other studies conducted in Fars, Kerman, and Isfahan during 1993–2001 reported the susceptibility of *Ph. papatasi* to DDT (18-21). Similarly, the susceptibility of *Ph. papatasi* to DDT, propoxur, and deltamethrin were confirmed in the studies conducted in Sabzevar, Isfahan, Baft, and Bam during 2002–2013 (20-21). This study was the first report on occurrence of resistance and under 'verification required' status of *Ph. papatasi* to DDT, deltamethrin, permethrin, and bendiocarb as well as susceptibil-

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ity to cyfluthrin in ZCL foci, west Iran. There are several novel investigations of the main vector-borne diseases in the country (28-31) and monitoring the susceptibility/resistance of related vectors is a vital responsibility of corresponding ministries (28-31).

Conclusion

The findings of the present study indicated *Ph. papatasi* resistance to DDT, occurrence of under 'verification required' status to bendiocarb, permethrin, and deltamethrin, and complete susceptibility to cyfluthrin.

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The authors declare that there is no conflict of interests.

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