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Baseline insecticide susceptibility data of *Phlebotomus papatasi* in Iran

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ABSTRACT

Background & objectives: Phlebotomine sandflies (Diptera: Psychodidae) play main role in the transmission of different forms of leishmaniasis in the world. *Phlebotomus papatasi* is the main vector of zoonotic cutaneous leishmaniasis (ZCL) in Iran. There are several control measures for vector control using different classes of insecticides. The aim of this study was to breed the sandflies which were collected from a hyperendemic focus of the disease in central Iran in the laboratory condition and to determine its baseline susceptibility to commonly used insecticides.

Methods: Sandflies were collected from the field and were reared in insectary. Susceptibility tests were carried out on their generation. Baseline susceptibility of sandflies to DDT and pyrethroids was evaluated based on LT_{50} values. A total of 1305 specimens were tested using different time intervals. The LT_{50} and LT_{90} values were measured according to the WHO standard tests. The results were plotted using probit analysis and regression lines.

Results: The results against female sandflies revealed the LT_{50} values of 1312.66, 253.66, 36.04, 9.38 and 6 sec to DDT (4%), permethrin (0.75%), deltamethrin (0.1%), cyfluthrin (0.15%) and lambda-cyhalothrin (0.05%), respectively. The figures for male sandflies were 1200.97, 310.10, 18.63, 6.08 and 0.77 sec respectively to the above insecticides.

Conclusion: The results of this study could help to provide a clue for implementation of currently used insecticides. Furthermore, a specific guideline is needed for monitoring and evaluation of insecticide susceptibility test against sandflies.

Key words Control; insecticides; Iran; *Phlebotomus papatasi*; zoonotic cutaneous leishmaniasis

INTRODUCTION

Leishmaniases are a group of vector-borne diseases transmitted by phlebotomine sandfly and caused by protozoan parasite of genus *Leishmania*[1–3]. Leishmaniasis is endemic in 98 countries or territories[4] — most of them are developing countries in tropical and subtropical parts of the world. In these areas, about 350 million population is at risk with about 12 million reported infections and about 60,000 deaths annually[3]. It is estimated that 1.5–2 million people are infected per year with 500,000 new cases for visceral leishmaniasis (VL) and 1–1.5 million for cutaneous leishmaniasis (CL), and of these only 600,000 cases are officially declared. The disease burden is estimated at 2,356,000 (946,000 in men and 1,410,000 in women) disability adjusted life years (DALYs); that show a significant ranking among communicable diseases[5]. Ninety percent of CL cases occur in 7 countries (Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria) and 90% of VL cases occur in rural and suburban areas in 5 countries (Bangladesh, India, Nepal, Sudan and Brazil)[2]. Different laboratories tried to rear sandflies in different parts of the world, each of them used special methods for feeding[6], temperature and other conditions[7–9]. Some of them encountered with some difficulties in rearing process like fungal contamination[10].

There are 56 species (32 *Phlebotomus* and 24 *Sergentomyia*) of phlebotomine sandflies in Iran but *Phlebotomus papatasi* is the main vector of ZCL[11]. Some researchers carried out susceptibility of sandflies in different parts of the world. They reported *P. papatasi* resistance to DDT in northern Bihar (India)[12] and Turkey[1,11]. In some parts of Rajasthan, *P. papatasi* was resistant to DDT but susceptible to dieldrin, malathion, fenitrothion and propoxur[13]. In other parts of Rajasthan this species was found resistant to DDT, dieldrin and propoxur while it was susceptible to malathion, fenitrothion and permethrin[13] while another study in Bihar showed that *P. argentipes* was resistant to DDT (4%) but susceptible to deltamethrin (0.05%)[14]. Italian populations of *P. perniciosus* and *P. papatasi* from Campania region and from Rome, respectively, were susceptible to the insecticides — (DDT (2%), lambda-cyhalothrin (0.06%) and permethrin (0.2%) as compared with the reference strain used[15]. In countries

where there are vector control operations for malaria, sandflies population could also be controlled. However, after stopping the use of insecticide, the number of leishmaniasis cases was increased[1,16]. In different areas of Iran, researchers carried out several tests. Results showed that in the ACL foci in northeastern part, *P. sergenti* was susceptible to deltamethrin[17] and DDT (4%) in Esfahan[18], and *P. papatasi* was susceptible to deltamethrin in Borkhar county, Esfahan province[19], susceptible to DDT in Badrood county[20], susceptible to DDT (4%), permethrin (0.25%), propoxur (0.1%) in Sabzevar[21], susceptible to DDT (4%) in Orzouiye county, Kerman province[22,11] and susceptible to DDT (4%) in Jarghuiyr county, Esfahan province and tolerant to DDT (4%) but susceptible to permethrin (0.75%), deltamethrin (0.1%), cyfluthrin (0.15%), and lambda-cyhalothrin (0.05%)[11]. Due to lack of enough information about susceptibility status of *P. papatasi* to recommended insecticides by WHO after testing the wild strain, we decided to test the laboratory-reared one against DDT and some current used pyrethroids[23].

MATERIAL & METHODS

Study area

This survey was conducted during the summer 2010 and sandflies were originated from the rural district of Badrood, Natanz county, Esfahan province, central Iran. This area is located in the foothills of the Karkas Mountains (altitude 3895 m). The area has a semi-desert climate. All the sandflies were collected from Matin Abad tourist camp that was situated in altitude of 978 m (33º45' N, 51º59' E).

Sandfly collection

Adults of *P. papatasi* were collected using hand aspirator and torch. The adults were caught from outdoor during dawn and dusk from parked car near to their breeding places. Collected sandflies were transferred to a cage with a hanging piece of wet cloth for supplying suitable humidity and were fed by a small amount of sucrose solution soaked cotton. All of them were placed in a plastic bag to remain wet and to maintain stable temperature situation. Subsequently, the cages were transported to Tehran sandfly insectary in the School of Public Health, Tehran University of Medical Sciences for sandfly maintenance and rearing[24]. Temperature was maintained by automated electric heaters and photoperiod of 14/10 H D/L was maintained in the insectary.

Sandfly rearing

After resting, fed and gravid wild-caught female adults were separated by aspirator and were released into individual pots according to Killick-Kendrick and Killick-Kendrick method[25] and were fed with honey solution (50%) and saturated sucrose. The pots were checked daily for hatching the eggs. The larvae (L_1) were fed with larval food, complex of rabbit food (palette) and rabbit feces without liver powder. In pupal stage the exuviate of L_4 and the tails exist at the end of pupae body. Before adding food for larvae, small amount of autoclaved sea sand was added. This is to prevent the larval movement in case of fungal infestation and were checked daily to take out fungi. Emerged adults were released in a new cage with wet cloth and sucrose solution (20%). Then the 3–10 days old adults were tested in a standard WHO susceptibility test method as described for mosquitoes. Identification of the adult females and males, was done based on Modi and Tesh method[26]. There are lot of problems in sandfly rearing such as fungal contamination, mite contamination, cannibalism and some anomaly in sandfly bodies. For instance, fungal contamination can trap larvae, especially I instar. For precaution of fungal growth, all materials and instruments were autoclaved at 121°C for 20 min. All the larval pots were disinfected. For mite protection, the insectary must has a routine washing program with warm water. Sometimes cannibalism could be observed in some pots especially at the I instar larvae. Sometime abnormality can be found in bodies at different stages.

Susceptibility tests

After some resting and feeding by sucrose solution (20%), sandflies were tested according to the standard method of WHO[27]. During the tests, the sandflies were released into the exposure tubes at different time intervals and then the mortality was counted after 24 h recovery period. During the holding time, the insects were supplied with cotton pad soaked in sucrose solution (20%). All the mortalities were corrected according to the results of control with Abbott's correction[28]. All the tests were excluded when the mortality was > 20% in the control group. After each test, all the dead and alive sandflies were transferred to 75% alcohol separately for mounting in Pouri's medium for species identification. Males and females were counted separately. Females and males at least 24 h after mounting, were identified using valid key[29, 30] and if there were any other species, all of them were excluded from the tests. Females of *P. papatasi* were identified by regular segments in spermatheca and network in pharyngeal armature.

The exposure time interval was between 7 and 3600 sec. At least 5 interval times were used to gain the mor-

tality between 5 and 95%. In each exposure time at least 4 replicates were used comprising 50–100 sandflies depending on the availability and the same age of the adults. The susceptibility tests were carried out on 1305 laboratory-reared *P. papatasi* (691 females and 614 males)

Procurement of insecticide papers and their concentration

Impregnated papers (DDT 4%, permethrin 0.75%, deltamethrin 0.1%, cyfluthrin 0.15%, lambda-cyhalothrin 0.05%) were procured from collaborating center of WHO in Malaysia.

Data analysis

The exposure time versus probit mortality were used according to Finney 1971. The Excel was used for data entering. HG4 software was used for drawing the graphs.

RESULTS

The results of tests against the laboratory-reared females and males are shown in Figs. 1 and 2, respectively. The results of susceptibility test against laboratory-reared

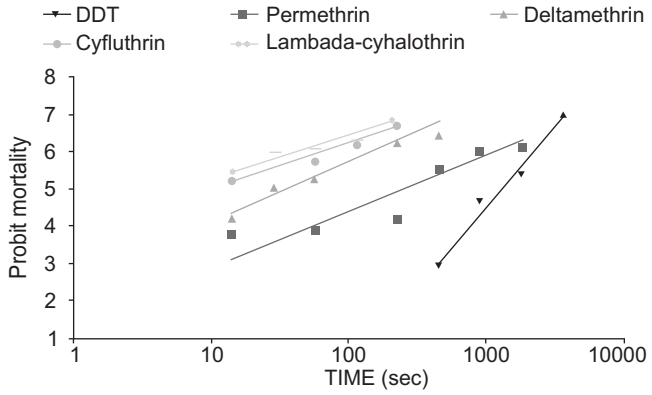

Fig. 1: Probit regression lines of different insecticides against laboratory-reared *P. papatasi* females.

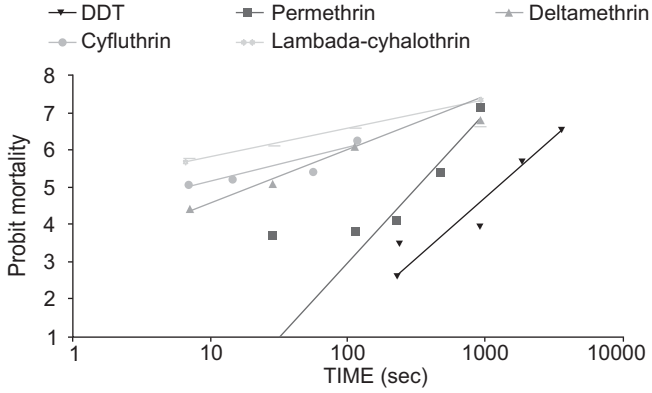

Fig. 2: Probit regression lines of different insecticides against laboratory-reared *P. papatasi* males.

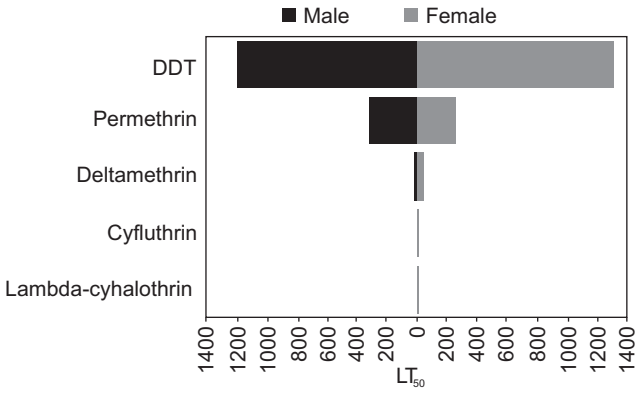

Fig. 3: LT_{50} values of different insecticides against laboratory-reared males and females *P. papatasi.*

female *P. papatasi* revealed LT_{50} values of 1312.66, 253.66, 36.47, 9.38, 6 sec to DDT (4%), permethrin (0.75%), deltamethrin (0.1%), cyfluthrin (0.15%) and lambda-cyhalothrin (0.05%) respectively. This data for males were 1200.97, 310.10, 18.63, 6.08 and 0.77 sec respectively to the above insecticides. The results showed that males were more susceptible than females to all the insecticides tested at LT_{50} level (Fig. 3).

DISCUSSION & CONCLUSION

The main measures for ZCL control in Iran are rodent control operation, using impregnated bednets and curtains with pyrethroids, the use of repellents, indoor residual spraying (IRS), and health education to the community during complex emergency situations, leishmanization is also recommended. Owing to the lack of information about the susceptibility of sandflies to different WHO recommended insecticides, the present study was undertaken. WHO susceptibility test recommended for mosquitoes was followed. The discriminative dose of DDT and pyrethroids were used in the tests. In accordance to WHO report[31], the bioassay results for malaria vectors were summarized in three resistant classes: susceptible with mortality rate 98–100%; possibly resistant (tolerant) with mortality rate between 97 and 80%; and resistant with mortality rate < 80%. Results of the test against laboratory-reared for females of *P. papatasi* at the LT_{50} values exhibited that males are more susceptible than females to all of the insecticides tested. Results of our tests against susceptible *P. papatasi* at LT_{50} level revealed that the females needs more time to be killed at the same concentration than males.

The time for mortality of sandflies was more than the pyrethroids. The high LT_{50} level of the vector to DDT is attributed to the long-term use of insecticides for malaria vector control in the region (from 1953 for up to 5 yr DDT

was applied as indoor residual spraying for malaria control in Badrood) that transmitted genetically to their progeny. In Esfahan province, several herbicides, fungicides and insecticides have been used for agriculture and veterinary pest control, including diazinon, malathion, azinphos-methyl, fenitrothion, metasystox, permethrin, carbaryl and cypermethrin. There are several reports of susceptibility of *Leishmania* vectors to different insecticides in Iran. For example, *P. papatasi* found susceptible to deltamethrin in Borkhar district, Esfahan province[19], and susceptible to DDT in Badrood district[20]. In Sabzevar district susceptible to DDT (4%), permethrin (0.25%) and propoxur (0.1%)[21]; and in Orzuie district in Kerman province susceptible to DDT (4%)[22]. *Phlebotomus sergenti* found susceptible to deltamethrin in Mashhad, northeast of Iran[17] and susceptible to DDT (4%) in Esfahan province[18]. There are several reports of reservoir hosts and thier control in Iran[32,33]. As we mentioned earlier[11], we recommend the same procedure in different parts of the world to pool the results and reach the unique conclusion about criteria for susceptibility status against *P. papatasi*. From the pooled results World Health Organization is able to provide a specific guideline for sandfly and this guideline will help the countries for monitoring and evaluation of insecticide resistance for implementation of control measures.

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