Susceptibility status of wild population of *Phlebotomus sergenti* (Diptera: Psychodidae) to different imagicides in an endemic focus of cutaneous leishmaniasis in northeast of Iran

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ABSTRACT

**Background & objectives:** Phlebotomine sandflies (Diptera: Psychodidae) transmit several important zoonotic diseases to humans and leishmaniasis is one of them. Two types of leishmaniasis, viz. visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) are endemic in Iran. The main vector of anthropotonic cutaneous leishmaniasis (ACL) is *Phlebotomus sergenti*. The aim of the present study was to determine the susceptibility status of wild strain of *P. sergenti* to different imagicides of DDT, bendiocarb and permethrin at the median lethal time, LT50 level.

**Methods:** Sandflies were collected from selected village in North Khorasan Province, northeast of Iran from indoors using CDC light-traps. Susceptibility test was carried out against DDT (4%), bendiocarb (0.1%) and permethrin (0.75%) for all the females according to WHO method, and mortality was calculated. Species identification was carried out using the morphological keys. Data were analysed using probit regression analysis to determine the LT50 and LT90 values.

**Results:** In total, 851 female *P. sergenti* sandflies were tested. LT50 values to DDT (4%), Bendiocarb (0.1%) and permethrin (0.75%) were 15.4, 19.2 and 6.3 min respectively. The values for LT90 were 51.1, 47.4 and 18.6 min respectively. The mortality rates for 1 h exposure time to DDT, bendiocarb and permethrin were 89.8 ± 1.4; 93.6 ± 1.4; and 95.6 ± 1.7%, respectively.

**Interpretation & conclusion:** The susceptibility studies revealed development of resistance against DDT (4%) in the wild strain of *P. sergenti* population. Monitoring and mapping of insecticide resistance in the region is recommended for vector control.

**Keywords** Insecticide resistance; Iran; leishmaniasis; *Phlebotomus sergenti*

INTRODUCTION

Phlebotomine sandflies (Diptera: Psychodidae) are important vectors of human leishmaniasis, affecting almost 98 countries of the world1. Iran is one of the 10 countries with the highest estimated cases of cutaneous leishmaniasis (CL) in the world1. In Iran, two common types of leishmaniases, viz. visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) are endemic. Visceral leishmaniasis is endemic in few foci of the country, including North Khorasan Province, and *Leishmania infantum* has been detected in vectors and reservoirs of the disease in this focus2-4. Cutaneous leishmaniasis is endemic in two forms, zoonotic cutaneous leishmaniasis (ZCL) and anthropotonic cutaneous leishmaniasis (ACL). ZCL is distributed in many rural areas of 17 out of 31 provinces (including North Khorasan) in Iran5-7. The main vector of ZCL is *P. papatasi* and *L. major* is the parasitic disease agent. Anthropotonic cutaneous leishmaniasis is endemic in at least eight provinces of Iran. The main vector of ACL is *P. sergenti*, and the disease agent is *L. tropica*, mainly distributed in large and medium-size cities of Iran5. More than 2831 cases of CL have been reported from several counties of this province in duration of eight years, from 2006 to 20137.

There are numerous reports on susceptibility status of phlebotomine sandflies to different insecticides in the world, that have used the discriminative dose of pesticides against malaria vectors, as described by WHO to determine the resistance of sandflies to insecticides8-21.
Some of these studies indicated that *P. sergenti* was susceptible to deltamethrin as well as DDT (4%) in the ACL foci in Mashad and Isfahan cities, in northeast and central Iran respectively. In the ZCL foci, *P. papatasi* was susceptible to DDT (4%) in Badrood county (central Iran), to DDT (4%), permethrin (0.25%) and propoxur (0.1%) in the Sabzevar (northeast of Iran), and to DDT (4%) in the Orzouyiye county (south of Iran).

In North Khorasan, DDT (75%) has been used as residual spraying since 1954 for malaria control. In recent years, several pyrethroids, carbamates and organophosphorus compounds are used for veterinary and agricultural pest control in many parts of the province (Ministry of Health, Iran).

Insecticide resistance threatens the effectiveness of control measures, and routine monitoring of insecticide resistance among natural populations of vectors further helps the authorities in early detection of resistance of vectors to insecticides, and improving effectiveness of operational control strategies. The present study was the first attempt to investigate the susceptibility status of phlebotomine sandflies to DDT, bendiocarb and permethrin on the wild population of *P. sergenti* in North Khorasan Province, Iran based on different exposure times. The *LT*$_{50}$ and *LT*$_{90}$ values were calculated using probit regression line. The comparison of different insecticides was carried out based on *LT*$_{50}$ and *LT*$_{90}$ values.

**MATERIAL & METHODS**

**Study area**

This survey was conducted during early-July to late August of 2015–16. Sandflies were collected from the Villages of Kohne Jolgeh, Maneh and Samalqan county, in North Khorasan Province, northeast of Iran where human VL and CL had been reported in the last 5 yr. The province is bordered by Turkmenistan in the north, Khorasan Razavi Province in the east and southeast, Semnan Province in the southwest and Golestan Province in the west. The study area is laid between 36°37′–38°17′ N latitude and 55°53′–58°20′ E longitude with an area of 28,434 km$^2$. The province comprises of eight counties with a population of 8,63,092 (2016 census). It has desert and mountainous areas and receives about 250 mm of rainfall annually. The City of Bojnurd is the center of the province. Kohne Jolgeh village is located near the Turkmenistan border, 130 km far from Bojnurd City and its population was 2900 in the year 2016.

**Sandfly collection**

Sandflies were collected from indoors including human dwelling and livestock shelters, using CDC light-traps from 2000 till 0200 hrs. The specimen collection was performed in accordance with the procedures approved by the Ethical Committee of North Khorasan University of Medical Sciences. The caught sandflies were transported immediately to the Research Center of Vector-Borne Diseases, North Khorasan University of Medical Sciences, Iran for carrying out the susceptibility tests. Collected sandflies were transferred in a wooden cage with a hanging piece of wet cloth for providing the suitable humidity. They were fed by a small amount of sucrose solution soaked cotton. In total, 851 field sandflies were collected and identified using appropriate keys.

**Procurement of insecticide impregnated papers and their concentration**

Impregnated papers with DDT (4%) (Batch No. DD186, Exp. date: July 2016); permethrin (0.75%) (Batch No. PE289, Exp. date: Sep 2015); and bendiocarb (0.1%) (Batch No. BE106, Exp. date: June 2016), as well as papers for control were supplied by the collaborating center of the World Health Organization in Penag, Malaysia.

**Susceptibility tests**

Collected sandflies were maintained under laboratory and appropriate climatic condition. Female sandflies were subjected to susceptibility tests according to the guidelines of WHO. Sandflies were exposed to impregnated papers (DDT, bendiocarb, permethrin) at different logarithmic times. They were provided 10% sucrose solution during the recovery period. The mortality was recorded after 24 h of recovery period. After each test, all the dead and alive sand flies were preserved in 96% ethanol for mounting in Puri’s media in order to identify the species using morphological keys. The exposure time was set between 3.75 and 60 min, and at least 5 log times were selected for estimation of lethal times. Out of the 851 field collected sandflies, 692 were used as treatment and 159 as control.

**Data analysis**

The exposure time vs probit mortality analysis was carried out according to Finney. The *LT*$_{50}$ and *LT*$_{90}$ values were estimated and the slope values of the regression lines were calculated.

**RESULTS**

In total, 692 female specimens were used for insecticide susceptibility tests. A total of 159 sandflies were
The results of the tests revealed the LT_{50} values to DDT (4%), bendiocarb (0.1%) and permethrin (0.75%) were 15.4, 19.2 and 6.3 min and the corresponding LT_{90} values were 51.1, 47.4 and 18.6 min (Table 1). The regression lines for mortality of *P. sergenti* exposed to the three insecticides used are plotted against exposure times (Fig. 1). Mortality rates calculated for *P. sergenti* after 1 h exposure to DDT (4%), bendiocarb (0.1%) and permethrin (0.75%) were 89.8 ± 1.4%, 93.6 ± 1.4% and 95.6 ± 1.7% respectively (Table 2).

### DISCUSSION

The susceptibility status of phlebotomine sandflies to DDT and other group of insecticides (bendiocarb, permethrin etc.) has been reported in several studies across the world. In Iran, some investigations have been carried out on *P. papatasi* but prior to this study, no reports are available on the susceptibility levels of phlebotomine sand flies (*P. sergenti*) from North Khorasan Province, one of the important CL foci in northeast of Iran. The present study is first attempt to reveal the susceptibility status of wild strain of *P. sergenti* to three conventional insecticides, i.e. DDT, bendiocarb and permethrin in this region.

According to the results of this study, permethrin 0.75% showed highest efficiency against *P. sergenti* at discriminative dose with mortality rate of 95.6 ± 1.7%, followed by bendiocarb (0.1%) and DDT (4%), with mortality rates of 93.6 ± 1.4% and 89.8 ± 1.4%, respectively. Resistance in *P. papatasi* has been reported for the first time in 1979 from Muzaffarpur district of Bihar, India [31] which was later supported in subsequent investigations [8]. In Iran, there are limited studies on insecticide susceptibility of phlebotominae sandflies. The result of these investigations have indicated the susceptibility of *P. papatasi* to different kinds of insecticide except tolerance to DDT in Isfahan Province, central Iran [24].

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**Table 1.** LT_{50} and LT_{90} values for *P. sergenti* to DDT (4%), bendiocarb (0.1%) and permethrin (0.75%), in North Khorasan Province, Iran (2016)

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>a</th>
<th>b ± SE</th>
<th>LT_{50} ± 95% CI</th>
<th>LT_{90} ± 95% CI</th>
<th>χ² (Heterogeneity)</th>
<th>χ² (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT (4%)</td>
<td>−2.916</td>
<td>2.4571 ± 0.264</td>
<td>12.8014</td>
<td>39.6321</td>
<td>2.048</td>
<td>7.81 (3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Bendiocarb (0.1%)</td>
<td>−4.176</td>
<td>3.2566 ± 0.723</td>
<td>16.4701</td>
<td>39.6321</td>
<td>16.752</td>
<td>7.81 (3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Permethrin (0.75%)</td>
<td>−2.187</td>
<td>2.7346 ± 0.423</td>
<td>4.4794</td>
<td>14.1533</td>
<td>3.518</td>
<td>7.81 (3)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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**Table 2.** Mortality parameters for *P. sergenti* when exposed to different insecticides using WHO’s method in a endemic focus of cutaneous leishmaniasis in North Khorasan Province, northeast of Iran, 2016

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>ET (min)</th>
<th>TST</th>
<th>ND</th>
<th>Mortality ± SD (%)</th>
<th>OPM</th>
<th>EPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT (4%)</td>
<td>3.75</td>
<td>36</td>
<td>2</td>
<td>5.6 ± 2.9</td>
<td>3.406</td>
<td>3.494</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>50</td>
<td>9</td>
<td>18 ± 8.1</td>
<td>4.005</td>
<td>4.205</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>49</td>
<td>27</td>
<td>55.1 ± 3.6</td>
<td>5.128</td>
<td>4.974</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>52</td>
<td>41</td>
<td>78.8 ± 1.7</td>
<td>5.801</td>
<td>5.713</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>59</td>
<td>53</td>
<td>89.8 ± 1.4</td>
<td>6.272</td>
<td>6.453</td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>51</td>
<td>1</td>
<td>2 ± 1.7</td>
<td>2.885</td>
<td>2.693</td>
</tr>
<tr>
<td>Bendiocarb (0.1%)</td>
<td>7.5</td>
<td>58</td>
<td>7</td>
<td>12.1 ± 4.2</td>
<td>3.907</td>
<td>2.693</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>40</td>
<td>5</td>
<td>12.5 ± 0.5</td>
<td>3.850</td>
<td>4.654</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>48</td>
<td>42</td>
<td>87.5 ± 0.5</td>
<td>6.150</td>
<td>5.634</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>78</td>
<td>73</td>
<td>93.6 ± 1.4</td>
<td>6.522</td>
<td>6.615</td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>21</td>
<td>4</td>
<td>19 ± 0.9</td>
<td>4.124</td>
<td>4.382</td>
</tr>
<tr>
<td>Permethrin (0.75%)</td>
<td>7.5</td>
<td>24</td>
<td>17</td>
<td>70.8 ± 4.2</td>
<td>5.548</td>
<td>5.173</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>19</td>
<td>15</td>
<td>78.9 ± 1.1</td>
<td>5.804</td>
<td>6.028</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>62</td>
<td>62</td>
<td>100 ± 0.0</td>
<td>6.702</td>
<td>6.851</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>45</td>
<td>43</td>
<td>95.6 ± 1.7</td>
<td>7.406</td>
<td>7.685</td>
</tr>
<tr>
<td>Control</td>
<td>159</td>
<td>1</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ET—Exposure time; TST—Total sandflies tested; ND—No. of dead sandflies after 24 h recovery period; SD—Standard deviation; OPM—Observed probit mortality; EPM—Expected probit mortality.

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Fig. 1: Comparison of regression lines, equations and LT_{50} of *P. sergenti* when exposed to DDT (4%), bendiocarb (0.1%) and permethrin (0.75%).
The reports on insecticide susceptibility status of *P. sergenti* are limited and there are no records of insecticide resistance till now. Similar studies in Egypt confirmed the susceptibility of *P. langeroni*, *P. papatasii* and *P. sergenti* to DDT, resmethrin, cyfluthrin, permethrin, bendiocarb and malathion. A study in Morocco, showed that wild populations of *P. sergenti* and *P. papatasii* were susceptible to lambdacyhalothrin, DDT and malathion. An earlier study on *P. sergenti* revealed that this species was susceptible to the deltamethrin and DDT among ACL foci in northeast, central and south Iran. All these studies have been conducted, according to the WHO guidelines for malaria vectors at the diagnostic dose. There are specific guidelines approved by WHO for diagnostic dose of mosquitoes, however, there are no such guidelines for phlebotominae sandflies; hence, trials were conducted, according to the results of LT50 and then compared with each other. However, the results of earlier studies were contradictory to this study.

CONCLUSION

The permethrin is efficient against *P. sergenti* at discriminative dose, followed by bendiocarb. For the first time, resistance against DDT was observed in wild strain of *P. sergenti* population in North Khorasan Province, Iran. These findings can be used as evidence for demonstrating the development of insecticide resistance in sandflies, indicating the necessity of systematic monitoring of resistance in sandfly populations.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

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