Bioassay evaluation of residual activity of attractive toxic sugar-treated barrier fence in the control of *Phlebotomus papatasi* (Diptera: Psychodidae)

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ABSTRACT

Background & objectives: *Phlebotomus papatasi* is the main vector of the zoonotic cutaneous leishmaniasis (ZCL) in Qom Province and many other provinces of Iran. Attractive toxic sugar baits (ATSB) treated barrier fence is one of the new methods for controlling the vectors such as sandflies. The present study was designed to evaluate the residual activity of ATSB-treated barrier fence that was used in control of *P. papatasi*.

Methods: Following the selection of villages in Markazi district of Qom Province, central Iran during 2015 for ATS and ASB (bait containing no active ingredient) methods; barrier fences on the ground in front of the rodent’s colony were installed. A total of four conical tubes were installed and fixed on surfaces of treated barrier net of dimension 25 × 25 cm at biweekly interval. In each conical tube, 10 sand flies were released and after 3 min of exposure they were transferred to sterile cups. After 24 h, the obtained results were recorded according to the survival and mortality rate of sandflies. These tests were carried out five days after the installation of barrier fences, and repeated every 15 days until the mortality rate decreased to 60–65%.

Results: The bioassay tests results showed that the mortality rate of *P. papatasi* on ATSB-treated barrier fence for 5, 15, 30 and 45 days after spraying was 100, 95.83, 88.18 and 66.67% respectively, which decreased to 50.83% after 60 days.

Interpretation & conclusion: Persistence and residual activity of the active ingredient of the bait in the hot and dry climatic conditions of Qom Province remained significantly effective for at most 45 days, which subsequently decreased at a high rate. Hence, every 45 days barrier fences need to be impregnated with ATSB bait. The method also appeared cost-effective and could be practical in implementation of vector control programmes against ZCL.

Key words  Attractive toxic sugar bait; bioassay test; Iran; *Phlebotomus papatasi*; Sandflies

INTRODUCTION

Phlebotominae sandflies (Diptera: Psychodidae) are biologic and proven vectors of human leishmaniases across the world¹-². These insects are also capable of transmitting some other pathogens to human such as *Bartonella* spp—the agent of bartonellosis (Carrion’s disease or Oroya fever), papatasi fever virus (family Bunyaviridae, genus Phlebovirus), Toscana virus, Chagres, Punta Toro, and Flaviviruses, Orbiviruses, Vesiculoviruses etc²-³. The phlebotominae sandflies of the subgenus *Adlerius* are well-known vectors for all types of visceral and cutaneous leishmaniasis (CL) in the Old World countries such as Iran². Among >800 species of these insects, only 98 species have role in disease transmission to human, including 42 *Phlebotomus* species in the Old World and 56 species of the *Lutzomyia* genus in the New World¹. Sandflies inhabit in human dwellings, stables, pet shelters, cracks on walls and cliffs, natural caves of mountainous areas, rodent burrows, fallen and decaying leaves in forests floor etc⁴. They have a wide geographical distribution ranging from Australia, through the Indian subcontinent to Central Asia, and Mediterranean countries in Europe, Africa and America⁵.

The sandfly vector species, *P. papatasi* is reported as the main vector of *Leishmania major* species in many countries such as Uzbekistan, Turkmenistan, Azerbaijan, Saudi Arabia, Jordan, Tunisia, Morocco and Iran⁶. This species is also recognized as the vector of *L. arabica* in Saudi Arabia and some arbovirus diseases in Iran and other countries in the world⁶-⁷. Moreover, *P. papatasi* is
the main vector of leishmaniasis in the Qom province of central Iran. So far, various methods have been tested for the control of sandflies in different areas of the world such as: the use of pyrethroid insecticides in insecticide-treated bed nets and indoor residual spraying (IRS), the use of lace, curtains and bed nets soaked with insecticide. Recently, a new promising strategy based on “attract and kill” phenomenon/process for controlling vectors like sandflies, called attractive toxic sugar bait (ATSB) method. In this method, vectors/sandflies are attracted to a surface or vegetation that coated/applied with ATSB containing oral toxins such as boric acid, which upon ingestion kills the insects. It has been used in recent studies in three forms ATSB-treated barrier fence, spraying of ATSB on vegetation, and the introduction of ATSB on bait stations which have been successful against vectors such as Anopheles, Culex, Aedes and sandflies in different parts the world. ATSB-treated barrier fence method has been exploited in the control of the populations of . A solution of the bait was prepared as described by with a little modification. The treatment sites consisted of 10% w/v brown sugar, 1% w/v boric acid and water (ATSB). A food dye, instead of boric acid, 10% w/v brown sugar and water (ASB) were used in the control site.

**ATSB-treated barrier fence**

In two villages of Koohsefid and Faraj Abad, rolls of semi rigid plastic net dimensioned 100 cm wide and suitably long enough depending on the extent of rodent colonies and 1 × 2 mm thick was used for building a bait barrier fence (Mesh size = 156 holes/ inch², 25 holes/cm², denier = 75). Strips of cotton cloth 5 × 60 cm² were connected transversally to the net, and their ends were folded around the margins and stapled. The strips were thus fixed to the net at intervals of 20 cm. After fixing the cloth strips, the net was rolled into a cylinder that was dipped into a bucket containing ATSB solution or solution without toxins. Initially, the number, location and extent of rodent colonies around the village were identified and characterized. Then, the nets of fences treated with ATSB and ASB were installed. This was done 500 m away from the distal houses of the outskirts of the village. In the experimental and control sites, barrier fences were installed on the ground in front of the colony of rodents, using about 150 cm metal rods that were driven 50 cm deep into the ground at the suitable distances and another 100 cm on the ground as a barrier to prevent the movement of sandflies towards the village. Residual efficacy rates of ATSB-treated barrier were evaluated using the World Health Organization protocols of bioassay test.

**Sandflies collection**

Sandflies for bioassay test were collected from indoor (bedroom, bathroom, toilets, hall and stables) fixed places in Mir Abad and Bagher Abad villages of Markazi district in Qom province from the first half of April 2015 to the first half of November 2015. To capture the sand flies, aspirators were used twice a month from sunset to sunrise 1 h before the bioassay test operation. The caught sandflies were transferred in holding tubes to the laboratory in Qom Health Center. Following the bioassay test, sand flies were mounted in Puri’s medium and identified after 24–72 h by considering the morphological characters. Then, these were counted and segregated by sex. The females were examined abdominally and the numbers of unfed, fresh-blood fed and gravid (semi-gravid)
sandflies were recorded. The results of physiological situation (parous and nulliparous) of sandflies were also recorded as shown in Table 1.

Bioassay test

The ATSB-treated barrier fence nets (25 × 25 cm) were removed from the installed barrier in the villages and were fixed by fiberglass sheets with a dimension of 25 × 25 cm and four quarters of a circle with equal intervals on the nets for stabling the cones (Fig. 1). The diameter of each circle was 9 cm, separated by a distance of 3 cm from one another, while the distance from the circles to the edge was 2 cm. Both the fiberglass sheets were connected with scotch tape and immobilized from one side. In order to enhance and improve the test accuracy and pollution prevention frameworks to various chemicals, disposable plastic sheets were installed on the internal surfaces with same dimensions, where the frame and the disposable plastic sheets were fixed to the internal surfaces framework by using scotch tape. The ATSB-treated nets (25 × 25 cm) were removed and placed into the plastic sheets, cones placed on the quarters of the circle of nets and the other fiberglass frame were fixed with clamps. Sandflies were immediately transferred to the laboratory, and after 1 h the sensitivity test was completed. For the determination of the residual effect of ATSB bait on barrier fence, bioassay test (cone test) was used in the treatment village by conforming to the World Health Organization protocol, using conical chamber at biweekly interval17. In the case of reduced residual effect of ATSB on barrier fences due to rainfall and so on, these were impregnated with ATSB solution again. In each conical tube, 10 sandflies were gently released in any cone at three replicates. Overall, in each biweekly interval, 120 sandflies were tested by using aspirator with a minimum exposure of 3 min on the treated surfaces. Three replicates of untreated surfaces were used as negative controls (ASB barrier fence nets) simultaneously. After 3 min exposure, these sandflies were transferred into clean paper cups and kept in suitable conditions (25°± 2°C and 80 ± 10% relative humidity) by placing a wet towel over the cups for 24 h. After the exposure time, both living and dead sandflies were transferred in netted cups to the laboratory, and the mortality was recorded after 24 h. If mortality rate of control tests was recorded between 5–20%, then the rates were corrected using Abbott’s formula. For the comparison of the residual activities in each sprayed surfaces, one-way ANOVA test was used. The criteria for the residual effect of tested insecticide were based on the mortality rates, and when this rate decreased to 60–65%17, the bioassays were stopped and the data were analyzed. To control the confounding effect on the results of deaths at all stages of caught sandflies released in each cone, the sandflies were caught again gently and carefully by using an aspirator. The obtained results were recorded according to the survival and mortality rate of sand flies. These tests were carried out from 5 days after spraying and repeated every 15 days until the mortality rate decreased to 60–65%17. In addition, the barrier fences were reviewed and repaired every 15 days.
RESULTS

The bioassay test results showed that the mortality rate of sandflies exposed to ATSB-treated barrier fence on Days 5, 15, 30 and 45 after spraying was 100, 95.83, 88.18 and 66.67%, respectively (Table 2). Subsequent persistence and residue effect of this method decreased, as the mortality rate after 60 days decreased to 50.83%.

Table 2. Results of bioassay tests on ATSB-treated barrier fence against Phlebotomus papatasi in Markazi district, Qom province 2015

<table>
<thead>
<tr>
<th>Days after application</th>
<th>No. of sandflies exposed to ATSB-treated barrier fence</th>
<th>No. of sandflies died after exposure to ATSB</th>
<th>Mortality rate ± S.E. of sandflies exposed to ATSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>120</td>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td>15</td>
<td>120</td>
<td>115</td>
<td>95.83 ± 4.35</td>
</tr>
<tr>
<td>30</td>
<td>120</td>
<td>106</td>
<td>88.18 ± 6.24</td>
</tr>
<tr>
<td>Monthly mean</td>
<td>120</td>
<td>113.67</td>
<td>91</td>
</tr>
<tr>
<td>45</td>
<td>120</td>
<td>81</td>
<td>66.67 ± 7.4</td>
</tr>
<tr>
<td>60</td>
<td>120</td>
<td>61</td>
<td>50.83 ± 65</td>
</tr>
<tr>
<td>Monthly mean</td>
<td>120</td>
<td>71</td>
<td>58.75</td>
</tr>
</tbody>
</table>

Table 3. Results of bioassay tests on ASB-treated barrier fence against Phlebotomus papatasi in Markazi district, Qom province 2015

<table>
<thead>
<tr>
<th>Days after application</th>
<th>No. of sandflies exposed to ASB-treated barrier fence</th>
<th>No. of sandflies died after exposure to ASB</th>
<th>Mortality rate ± S.E. of sandflies exposed to ASB</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>120</td>
<td>8</td>
<td>6.67 ± 2.28</td>
</tr>
<tr>
<td>15</td>
<td>120</td>
<td>6</td>
<td>5.00 ± 4.24</td>
</tr>
<tr>
<td>30</td>
<td>120</td>
<td>10</td>
<td>8.34 ± 8.75</td>
</tr>
<tr>
<td>Monthly mean</td>
<td>120</td>
<td>8</td>
<td>6.67</td>
</tr>
<tr>
<td>45</td>
<td>120</td>
<td>8</td>
<td>6.67 ± 10.35</td>
</tr>
<tr>
<td>60</td>
<td>120</td>
<td>14</td>
<td>11.67 ± 7.84</td>
</tr>
<tr>
<td>Monthly mean</td>
<td>120</td>
<td>11</td>
<td>8.92</td>
</tr>
</tbody>
</table>

But the mortality rate of exposed sandflies to ASB-treated barrier fence after 5, 15, 30, 45 and 60 days were 6.67, 5, 8.34, 6.67 and 11.67%, respectively (Table 3). The activity of P. papatasi in the region started from May and ended in November, with two peaks observed in early June and late July (Fig. 2).

DISCUSSION

This is the first formal report on the field evaluation of ATSB-treated barrier fence active ingredient in Qom province, central Iran. The control method employed in this study is quite considerable, as both male and female phlebotomine sandflies, like other biting flies, require sugar nectar from plants and shrubs for survival. Hence, this behavioral characteristic of the insect may be considered to control the sandflies. ATSB has been successfully used in vector control programs, especially in the control of sandflies which are leishmaniasis-transmitting vectors, in three applicable forms: (i) spraying of ATSB on vegetation; (ii) ATSB presented on bait stations; and (iii) ATSB-treated barrier fence. In some studies, ATSB consisted of 10% w/v brown sugar, boric acid 1% w/v and water. Boric acid (H₃BO₃), also called hydrogen borate, is a weak, monobasic Lewis acid of boron often used as an antiseptic, insecticide, flame retardant, neutron absorber, or precursor of other chemical compounds. It exists in the form of colorless crystals or a white powder that dissolves in water. Boric acid is a safe oral toxin, and an effective stomach poison for insects. After introducing it to ATSB, sandflies ingest the toxic solutions and are killed. Boric acid may be much cheaper and locally made.

One of the important endemic vector-borne diseases in Qom province is CL. Based on epidemiological and molecular studies, the type of CL prevalent in the districts of the Qom province is ZCL. The two endemic foci of ZCL are Kahak and Markazi district. Previous studies have revealed that, in these ZCL foci, usually Leishmania-infected sandflies move from rodents burrow towards human dwellings in order to continue biting and feeding on human sources.

In the present study, ATSB-treated barrier fence was used in the study villages located in close proximity to the frontal view of rodent borrows facing toward human dwellings. The P. papatasi species is the main sandfly vector of ZCL in Iran. The residual efficacy would help in the determination of next repeated impregnation. In many previous studies insecticides have been applied for phlebotomine sandflies control such as insecticide-treated bed nets, bioassay test has been used and tested. Based on the bioassay test, ATSB-treated barrier fence...
in Qom province climate (arid climate) was found effective for 45 days at most. Therefore, every 45 days barrier fences need to be impregnated with ATSB bait. The mortality rate of sandflies exposed to ATSB treated barrier fence on Days 5, 15, 30, 45 and 60 was 100, 95.83, 88.18, 66.67 and 50.83%, respectively. But the mortality rate of exposed sandflies to ASB-treated barrier fence after 5, 15, 30, 45 and 60 days was 6.67, 5, 8.34, 6.67 and 11.67%, respectively. This value of the mortality rate was both normal and expected. Possibly, the mortality rate among sandflies which was exposed to ASB may be attributed to the relocation and transportation of sandflies. Because *P. papatasi* activity started from May and ended in November in the study region, and residents received most infected bites during July to September, a majority of patients come to clinics for treatment from October until late December. ATSB method correlates well with other sandfly control methods that create a barrier between humans and sandflies such as insecticide-treated bednets (ITNs) or indoor residual spraying (IRS). Also residual efficacy of 45 days can be cost-effective and affordable in comparison is to control methods. Although the residual activity of the active ingredient of the bait in the climatic conditions of Qom province is effective for at most 45 days, which subsequently decrease at a higher rate. Hence, every 45 days barrier fences need to be impregnated with ATSB bait. So, in ZCL foci, ATSB can be one of the integrated CL vector control program. Based on the results of study, this method is cost-effective and it can work as a new tool for vector control that can be integrated with other control methods in integrated vector management (IVM) in ZCL foci such as in Qom province.

**CONCLUSION**

The results indicated that persistence and residual activity of the active ingredient of the bait in the climatic conditions of Qom province is effective for at most 45 days, which subsequently decrease at a higher rate. Hence, every 45 days barrier fences need to be impregnated with ATSB bait. So, in ZCL foci, ATSB can be one of the integrated CL vector control program. Based on the results of study, this method is cost-effective and it can work as a new tool for vector control that can be integrated with other control methods in integrated vector management (IVM) in ZCL foci such as in Qom province.

**Conflict of interest**

The authors declare no conflict of interest.

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