

## Susceptibility of phlebotomine sandflies (Diptera: Psychodidae) collected in the field, to alpha-cypermethrin in four municipalities endemic to leishmaniasis

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### ABSTRACT

Although the chemical control against leishmaniasis began in 1953 in Brazil, little information is available on how this strategy has affected populations of phlebotomine sandflies in the field. The objective of this study was to analyze the susceptibility profile of four populations of phlebotomine sandflies to the insecticide alpha-cypermethrin. Sandflies collected in field in four Brazilian municipalities were evaluated using CDC bottles in different concentrations of alpha-cypermethrin. A total of 1,186 phlebotomine sandflies were used in the bioassays. The LD<sub>50</sub> ranged from 1.48 to 2.57 ug/mL in the field populations. For a dose of 5 ug/mL of alpha-cypermethrin, the LT<sub>50</sub> and LT<sub>95</sub> ranged from 17.9 to 27.5 minutes, and LT<sub>95</sub> from 39.7 to 61.5 minutes, respectively. All the populations studied were highly susceptible to the insecticide alpha-cypermethrin. Routine studies are needed to detect changes in sandflies susceptibility to insecticides.

**KEYWORDS:** Phlebotominae. Leishmaniasis. Insecticide resistance. Bioassay. Alpha-cypermethrin.

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Visceral leishmaniasis (VL) and American Cutaneous Leishmaniasis (ACL) are considered zoonoses, and are present in 98 countries throughout the Americas, Europe, Africa and Asia; VL is endemic in 75 countries, and ACL in 87<sup>1</sup>. Despite the vector control activities undertaken by the Ministry of Health since 1953, in recent years the leishmaniasis have expanded across Brazil to a worrying extent. Between 2007 and 2017, 41,173 confirmed cases of VL and 232,989 cases of ACL were reported in Brazil<sup>2</sup>. This fact leads to a deeper reflection about the true effectiveness of the insecticide used on Brazilian phlebotomine sandfly populations. It should be noted that the insecticide resistance in sandflies has been reported in some old-world countries, related to mutations in sodium channel proteins and/or enzymatic alterations which have still not yet been widely investigated or understood<sup>3</sup>. This information is even more important in areas in which different overlapping programs are used to control endemic diseases, acting simultaneously but in a disjointed manner. In practice, this situation translates the exposing of the sandflies to much higher doses of insecticides than those recommended for controlling this vector, with the potential of selecting resistant individuals<sup>4,5</sup>.

The Ministry of Health of Brazil, since 2003<sup>6</sup>, recommends the spraying of home units with 40 mg/m<sup>2</sup> of alpha-cypermethrin (BASF, Brazil) for the control of sandflies in areas with ACL and VL transmission, in two different spray cycles with intervals of 120 days, the first cycle after the rainy season<sup>7</sup>. However, no assessment of the

impact of this insecticide on the susceptibility of Brazilian sandfly populations from endemic areas has been carried out.

In this context, the objective of this study was to describe the susceptibility/resistance profile of Brazilian sandfly populations to the pyrethroid insecticide alpha-cypermethrin.

The sandflies were captured in Minas Gerais State, in the localities of Salgado Filho/Belo Horizonte (S 19° 56' 56.1"; W 043° 58' 38.0"), Coqueiro/Januaria (S 15° 25' 55.6"; W 044° 24' 39.7"), and Morrinhos/Montes Claros (S 16° 44' 04.7"; W 043° 51' 42.3"), and in the Goiás State, in the locality Jardim Babilonia/Pirenópolis (S 15° 51' 50.8"; W 048° 58' 14.2") between April and October 2015. The leishmaniasis are endemic in these areas, with a significant number of human and canine cases of VL and ACL. In addition, in these areas, the State Leishmaniasis Control Programs carry out continuous and systematic applications of alpha-cypermethrin for controlling the high density of sandflies. Light traps were installed, in the intra and peridomicile, in the municipalities on three consecutive nights (6:00 pm-7:00 am). The captured sandflies were maintained for two hours in the dark with a 30% sucrose solution for acclimatization prior to the conduction the bioassays.

The bioassays were performed with controlled temperature and humidity (26 ° C and 48%) and were carried out by CDC bottle bioassay methodology<sup>8</sup>. For this, we followed the WHO guideline which recommends adopting the discriminating concentration to determine the concentration that causes 100% mortality of adults; 250 mL glass bottles (Wheaton Glass Media Bottles, USA) were impregnated with 1 mL of alpha-cypermethrin dissolved in acetone solution (P.A Merck, Brazil), in four concentrations (3, 5, 7 and 9 µg/bottle) prepared from the stock solution 24 h before the test. Sandflies were exposed to the insecticide for up to 60 min, with the mortality rate recorded every 10 min. Doses of 3, 5, 7 and 9 µg/mL were used to measure lethal doses (LT<sub>50</sub> and LT<sub>95</sub>), while the dose of 5 µg/mL was used to estimate the lethal times (LT<sub>50</sub> and LT<sub>95</sub>) for the four populations analyzed. An average of 20 specimens was tested per bottle without distinction by sex and in equal proportions (1:1). The insects in the control group were only exposed to acetone. A total of 60 sandflies were used as controls. The insects were collected in intra and peridomicilar areas of

Coqueiro, Morrinhos and Jardim Babilonia. In Salgado Filho, the control was carried out with 30 insects. The tests were repeated three times. Insects which demonstrated a change in their ability to fly and/or immobility were considered dead. Next, the sandflies were transferred to containers without insecticide holding 30% sucrose solution, which was offered *ad libitum*; they were kept there for 24 h under controlled temperature and humidity conditions to evaluate their potential recovery from the knockdown effect (Kdr) characteristic of pyrethroid insecticides. Susceptibility was analyzed using the JMP software, version 10 for Windows (SAS Institute I, Cary, NC, USA)<sup>9</sup>.

A total of 1,186 phlebotomine sandflies were used in the bioassays. *Lutzomyia longipalpis* was the most frequently captured species in all the localities (88%), followed by *Lu. ischnacantha* (6%), *Lu. renei* (3%), *Nyssomyia whitmani* (1%), *Evandromyia lenti* (0.8%), *Micropygomyia villelai* (0.6%), *Ev. (Barrettomys) série Cortelezzii* (0.2%), *Ev. bacula* (0.2%), and *Brumptomyia avellari* (0.2%).

Although the initial focus of this research was the susceptibility/resistance of *Lu. longipalpis* to the pyrethroid insecticide alpha-cypermethrin, other species were seen to occur in lower proportions. Notably, among them there were *Ny. whitmani*, a vector of *Leishmania braziliensis* in Brazil. It was not possible to select only *Lu. longipalpis* to conduct the tests as the taxonomic identification was only performed after the biological bioassays were complete. This identification depends on the preparation and mounting of dead specimens followed by examination of their external and internal structures<sup>10</sup>; in other words, only after exposure to the insecticide could the species be correctly identified.

In Brazil, the Ministry of Health recommends a single strategy for the chemical control of phlebotomine sandflies in the entire country<sup>7</sup>; this allows us to assume that all urban species are subject to the same pressure from insecticides. Bearing this in mind, we suggest dose-response assays for species that occur in the intra and peridomicile to estimate differences between the susceptibility of each species.

Phlebotomine populations from Salgado Filho, Coqueiro, Morrinhos and Jardim Babilonia showed high susceptibility to the pyrethroid insecticide alpha-cypermethrin. The LD<sub>50</sub> and LD<sub>95</sub> for the field populations are shown in the [Table 1](#).

**Table 1** - Estimated lethal dose of different populations of sandflies exposed to alpha-cypermethrin.

Population	n	LD <sub>50</sub> ug/mL (95% CI)	LD <sub>95</sub> ug/mL (95% CI)
Salgado Filho	134	2.05 (0.02 - 3.35)	7.01 (5.95 - 9.56)
Coqueiro	340	2.57 (2.07 - 2.84)	4.14 (3.75 - 5.32)
Morrinhos	463	1.48 (0.22 - 2.13)	3.39 (2.69 - 4.30)
Jardim Babilonia	249	2.45 (1.11 - 3.16)	5.80 (4.61 - 11.04)

LD = lethal dose in micrograms per bottle; 95% CI = 95% confidence interval

In the field populations,  $LT_{50}$  ranged from 17.9 min to 27.5 min, and  $LT_{95}$  from 39.7 min to 61.5 min (Table 2). In the mortality analysis performed 24 h after exposure to alpha-cypermethrin, no recovery from the knockdown effect was observed. However, within the bottles the sandflies exposed to the pyrethroid were consistently seen to lose their legs; this phenomenon was not seen in the control group, in which all the insects remained alive until the end of the experiment. The loss of legs is seen as a sublethal effect for sandflies, making it impossible to dislocate and consequently feed and transmit *Leishmania*<sup>11</sup>.

**Table 2** - Estimated lethal time of different populations of sandflies exposed to 5 ug/mL alpha-cypermethrin.

Population	$LT_{50}$ (95% CI)	$LT_{95}$ (95% CI)
Salgado Filho	24.2 (20.1 - 29.1)	39.7 (33.3 - 47.3)
Coqueiro	27.5 (23.8 - 31.7)	61.5 (53.6 - 70.6)
Morrinhos	19.6 (17.0 - 22.6)	47.2 (41.4 - 53.8)
Jardim Babilonia	17.9 (13.3 - 24.2)	40.9 (30.9 - 54.2)

Lethal time (LT) was expressed in minutes;  $LT_{50}$  = 50% lethal time;  $LT_{95}$  = 95% lethal time. 95% CI = 95% Confidence Interval.

In Brazil, only five studies evaluated the susceptibility of sandflies to insecticides. In the first study, Falcão<sup>12</sup> tested *Lu. longipalpis* resistance profile from Gruta da Lapinha, in Minas Gerais for organochlorines DDT and Dieldrin. In 1988, Falcão *et al.*<sup>13</sup> evaluated this same population for the pyrethroid deltamethrin. In both studies, sandflies were susceptible. Based on this information and the fact that the Lapinha region had no contact with the insecticide, Alexander *et al.*<sup>11</sup> considered this population as a reference in a study that compared it with sandflies from Montes Claros, also in Minas Gerais (location impacted by chemical control). In this experiment, the authors reported a reduction in the susceptibility of the sandflies of Montes Claros to the permethrin and deltamethrin pyrethroids. This was the first record of an insecticide tolerance of sandfly species in South America. Pessoa *et al.*<sup>14</sup> after testing sandflies from Gruta da Lapinha indicated this population as a susceptible reference lineage, as it complies with the requirements of The Pan American Health Organization<sup>14</sup>. More recently, González *et al.*<sup>15</sup> tested populations of *Lu. longipalpis* caught in the region of Aracatuba. The results demonstrated the susceptibility of these populations to lambda-cyhalothrin and deltamethrin pyrethroids.

The data on susceptibility, presented here, demonstrate the importance of monitoring populations of sandflies to insecticides, so that detection and control measures can be taken in a timely manner in face of the emergence of resistance in Brazil.

For the tests, sandflies with high physical vigor and visibly healthy were selected, but the use of the CDC trap may have influenced the mortality. The captured species may have suffered injuries caused by the propellers of the apparatus, and this fact can be considered as a limitation of this study.

Populations of phlebotomine sandflies from the localities of Salgado Filho/Belo Horizonte, Coqueiro/Januaria, Morrinhos/Montes Claros and Jardim Babilonia/Pirenópolis are susceptible to alpha-cypermethrin and *Lu. longipalpis* was the predominant species. Considering the importance of bioassay findings in guiding leishmaniasis vector control strategies, laboratory tests should be routinely performed so that changes in the susceptibility of sandflies to insecticides can be timely detected.

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## AUTHORS' CONTRIBUTIONS

Douglas de Almeida Rocha: collection, carrying out the experiments, analyzing the results and writing the study; Andrey José de Andrade: project design, results analysis, text review; Luciana Moura: analysis of results and statistical analysis of the study; Nelder de Figueiredo Gontijo: experimental design and validation of susceptibility bioassays; Grasielle Caldas D'Ávila Pessoa: project design, analysis of results, text review; Marcos Takashi Obara: project design, study writing and text review.

## CONFLICT OF INTERESTS

None to declare.

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