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Solid phase microextraction, sand flies, oviposition pheromones, plaster of Paris and siloxanes—What is in common?

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ABSTRACT

Sand flies are natural hosts of various microorganisms. Due to their epidemiological importance, sand fly colonies are kept in laboratories to be studied in terms of their biology and vector/host/parasite interactions. In order to investigate the presence of oviposition pheromones in *Nyssomyia neivai*, experiments using Solid Phase Microextraction (SPME) were performed. However, siloxanes which is an external class of contamination, present in breeding containers made by plaster used to maintain sand flies in colonies, may be hindered the experiments.

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1. Introduction

Solid Phase Microextraction (SPME) is a solvent-free method for the extraction of volatile and semi volatile compounds first used for detecting traces of organic compounds in liquids analyses (Pawliszyn, 1995; Horák et al., 2009). Nowadays it has been used to find sexual pheromones in many insects orders, as Lepidoptera (Frérot et al., 1997), Coleoptera (Fujiwara-Tsujii et al., 2012), Diptera (Farine et al., 2012) and Hemiptera (May-Concha et al., 2015). However, SPME for detecting oviposition pheromones in insects has been found only in Hemiptera (Verheggen et al., 2008).

Oviposition pheromones studies in Phlebotomine sand flies (Diptera: Psychodidae) have been restrict to *Lutzomyia longipalpis*, vector of visceral leishmaniasis. Experiments with *Lu. longipalpis*, using hexane extract, showed a positive response to eggs (Dougherty et al., 1993; Elnaiem and Ward, 1991) and latter on, dodecanoic acid was identified as the oviposition pheromone (Dougherty and Hamilton, 1997). Nevertheless, there are over 900 sand flies species identified without any other oviposition pheromone detected.

Nyssomyia neivai, is a species strongly incriminated to be a vector of *Leishmania (Viannia) braziliensis*, etiological agent of American cutaneous leishmaniasis (Córdoba-Lanús et al., 2006; Casanova et al., 2009; Marcondes et al., 2009) and some previous behaviour experiments in our laboratory (data not shown) indicated a greater oviposition rate where previous eggs were placed. Some experiments were performed to detect a possible oviposition pheromone present in this sand fly species. Solid Phase Microextraction (SPME) and gas chromatography were used but high peaks of a contaminant, siloxane, made the analysis unfeasible. After some attempts we suspect that the plaster of Paris, the base of the sand flies breeding pots, which are used in routine to breed sand flies in laboratory conditions (Modi and Tesh, 1983), might have been the contamination source. Our aim is report and discuss the non-viability of using this method in such specific context.

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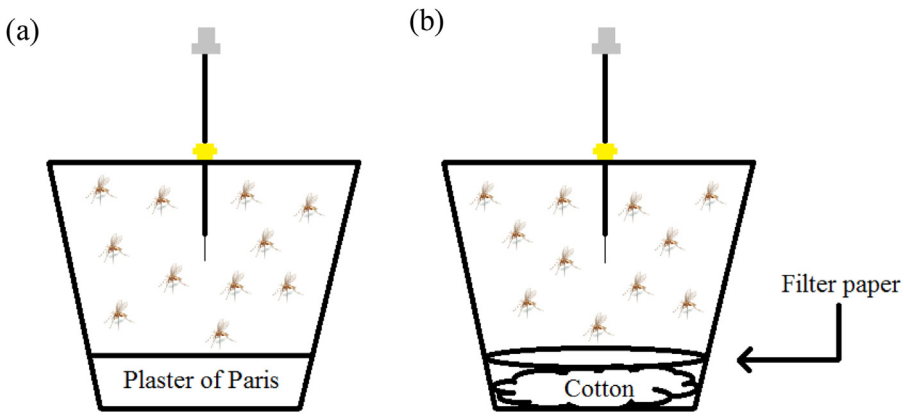

Fig. 1. Extractions using SPME methodology. Fiber in a pot with sand flies and plaster of Paris in the bottom as substrate (a), fiber in a pot with sand flies and cotton plus filter paper in the bottom as substrate (b).

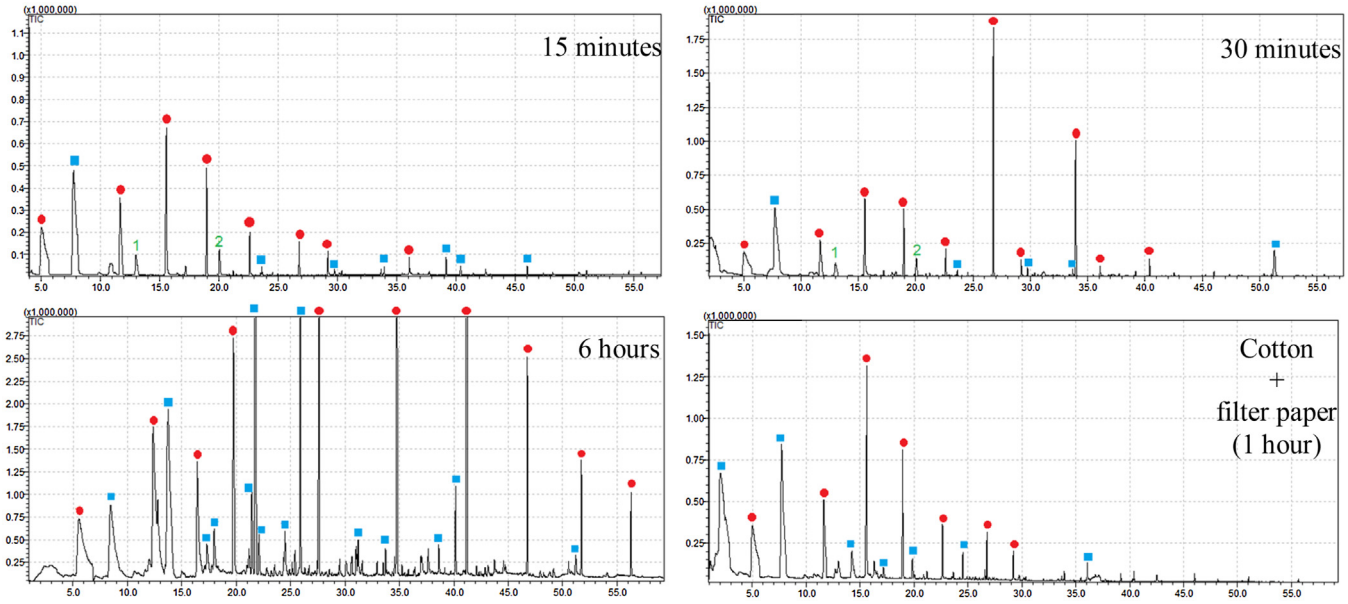

Fig. 2. Chromatograms of different times of extraction with plastic of Paris and extraction of the pot with cotton and filter paper in the bottom as a substrate, with all showing contamination of siloxanes (●). Peaks with low similarity or no identification are represented by (■), and peaks 1 and 2 were identified as 2-ethyl-1-hexanol and naphthalene, respectively.

2. Material and methods

Sand flies were obtained from a colony maintained at Biological Sciences Department of School of Pharmaceutical Sciences, Araraquara, São Paulo state, Brazil (Goulart et al., 2015).

All experiments were performed using SPME to extract possible oviposition volatiles.

2.1. First trial

The bottom of polystyrene tubes (15 mL) was covered with quick-drying plaster of Paris (Fortaleza®), a round piece of its cover was removed and a mesh was glued in the empty space. Sandflies were inserted into the vials and the top was closed. A piece of cotton soaked with 30% sugar solution was placed over the mesh to allow the insects to feed. A piece of Parafilm® paper was used to seal the top and prevent the volatiles to disperse. Three treatments were performed: a) three couples of sand flies – females without blood feeding; b) three couples – females after blood feeding; c) and empty vials (as blank control). After four days, time for oviposition, silica fibers (50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane – DVB/CAR/PDMS – Sigma Aldrich®) were inserted to extract volatiles. The exposition time to extraction was 15, 30 and 45 min for the three treatments, in order to detect the better time. The fibers were inserted in Gas Chromatography–Mass Spectrometry (GC–MS, QP2010 Plus) for analysis on an ZB-5 column (30 m × 0.25 mm, 0.25 μm; temperature program: 50 °C for 3 min; 50–210 °C at 3 °C/min; 210 °C for 3 min) and the tests were carried out in triplicates (Fig. 1a).

Second trial

Considering the low number of eggs of the first experiment (49 eggs) the same procedure was repeated but at this time six couples were used and also the extraction time was increased to six hours (Fig. 1a).

Third trial

One thousand and two hundred eggs were placed inside a 2 mL vial. As a control, thin pieces of plaster of Paris, where the females laid the eggs, were transferred to another vial. Three fibers were