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NOTA BREVE/ SHORT NOTE

ENTOMOLOGÍA

TOXIC ACTIVITY OF PYRETHROIDS IN *Lutzomyia longipalpis* (DIPTERA: PSYCHODIDAE) FROM MAGDALENA RIVER BASIN, COLOMBIA

Actividad tóxica de piretroides en *Lutzomyia longipalpis* (Diptera: Psychodidae) procedente del Valle del Magdalena Medio, Colombia

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ABSTRACT

The study aimed to determine the toxicity of lambda-cyhalothrin, alpha-cypermethrin, and deltamethrin in *L. longipalpis*, through concentration-mortality bioassays. The test here was performed following WHO guidelines, but instead of using exposure WHO recipients and impregnated papers, 250 ml Wheaton glass bottles treated with 1 ml of insecticide solution were used. Batches of ten females of *L. longipalpis* were exposed to five concentrations of each pyrethroid that caused between 5 and 100 % mortality in this species. After 1 h of exposure, the females were transferred to observation recipients, and mortality was recorded 24 h later. The lethal concentrations (µg/ml) that killed 50 and 95 % (LC_{50} and LC_{95}) of the exposed *L. longipalpis* females were 0.05 and 0.86 for lambda-cyhalothrin, 0.24 and 3.62 for alpha-cypermethrin and 0.53 and 4.72 for deltamethrin. Based on the LC_{50} obtained, lambda-cyhalothrin is the most toxic pyrethroid for *L. longipalpis*, followed by alpha-cypermethrin and deltamethrin. It is expected that these data may be useful in studies on the effects of sub-lethal concentrations of the three pyrethroids on the behavior of *L. longipalpis* and studies on the vector susceptibility to these pyrethroids.

Keywords: Biological assay, Colombia, insecticides, leishmaniasis, Psychodidae.

RESUMEN

El objetivo del estudio fue determinar la toxicidad de los piretroides lambdacialotrina, deltametrina y alfacipermetrina en *L. longipalpis*, a través de ensayos concentración-mortalidad. Los ensayos se hicieron siguiendo los lineamientos de la OMS, pero en lugar de los recipientes de exposición y de los papeles impregnados de la OMS, se utilizaron botellas de vidrio Wheaton de 250 ml tratadas con 1 ml de solución de insecticida en alcohol absoluto. Grupos de 10 hembras de *L. longipalpis* sin alimentación sanguínea fueron expuestos a cinco concentraciones de cada piretroide, que causaron entre el 5 y 100 % de mortalidad. Pasada una hora de exposición, las hembras se trasladaron a los recipientes de observación y la mortalidad se registró 24 h después. Las concentraciones (µg/ml) que mataron el 50 y el 95 % (CL_{50} y CL_{95}) de las hembras expuestas de *L. longipalpis* fueron de 0,05 y 0,86 para la lambdacialotrina, 0,24 y 3,62 para la alfacipermetrina y 0,53 y 4,72 para la deltametrina. Basados en las CL_{50} obtenidas, la lambdacialotrina fue el piretroide con mayor toxicidad para *L. longipalpis*, seguido por la alfacipermetrina y la deltametrina. Se espera que estos datos puedan ser útiles en estudios de los efectos de concentraciones sub-letales de los tres piretroides en el comportamiento de *L. longipalpis* y en estudios de la susceptibilidad del vector a los mismos.

Palabras clave: Bioensayo, Colombia, insecticidas, leishmaniasis, Psychodidae.

INTRODUCTION

In the Americas, *Lutzomyia longipalpis* (Lutz and Neiva, 1912) is the main vector of *Leishmania infantum*, the causative agent of visceral leishmaniasis, which is the most severe form of leishmaniasis. Vector control is one of the most important components of disease management, seeking to reduce the exposure of individuals to the infective bite of sandflies, either by reducing the human-vector contact or the density of vectors in specific habitats (Molyneux, 1993). Chemical insecticides are used in different measures, and one of the most important goals is to reduce the age of the natural population of vectors reducing colonization efficiency by an etiologic agent that makes the insect a vector. For *L. longipalpis* control, different measures have been evaluated, especially chemical measures, and most of them include pyrethroid insecticides and involve spraying of the human dwellings and surfaces of animal shelters (Kelly *et al.,* 1997; Barata *et al.,* 2011), insecticide-treated nets (Courteney *et al.,* 2007), dog collars (David *et al*., 2001) and pheromone baits (Bray *et al*., 2010). The efficacy of those measures against *L. longipalpis* is generally high and spraying over vector resting surfaces with residual action insecticides, including pyrethroids, is recommended in visceral leishmaniasis foci in Colombia (Ministerio de la Protección Social *et al*., 2012) and Brazil (Ministério da Saúde, 2013). However, baseline information about the toxic activity of pyrethroids in *L. longipalpis* is limited.

Insect populations have normal response intervals to each insecticide. These intervals are determined by evaluating, under controlled conditions, increasing concentrations of the toxic substance (stimulus), and after a certain exposure time, response variables such as the mortality are evaluated. Based on these concentration-response tests, it is possible to infer the toxic concentration that kills a determinate percentage of exposed insects, for example to 50 % or 95 %, as corresponding to the lethal concentrations 50 (LC_{50}) and 95 (LC_{95}), respectively (Lagunes-Tejeda *et al.*, 2009). Lethal concentrations (with their confidence intervals) are quantitative expressions of the toxicity of an insecticide for a given species allowing to establish comparisons of the toxic activity of two or more insecticides, for which a lower value of LC_{50} will indicate greater toxicity, and this knowledge may be useful to select insecticides during an intervention. Also, these concentrations are a measure of the susceptibility of a species to an insecticide under experimental conditions (Hubert, 1980).

By the other hand, the susceptibility is the tolerance range in a population to an insecticide compared with 1) a susceptible strain (e.g., *Aedes aegypti* Rockefeller strain) or 2) baseline susceptibility indicators of this population.

This study aimed to determine the toxicity of the pyrethroids lambda-cyhalothrin, alpha-cypermethrin, and deltamethrin in *L. longipalpis* through concentration-mortality tests. The first two pyrethroids are recommended for the residual treatment of the outdoor surfaces of dwellings for visceral leishmaniasis vector control, and most of the studies evaluating treated materials against *L. longipalpis* include one of these two insecticides (Feliciangeli *et al.*, 2003, Romero and Boelaert, 2010). In addition, all three pyrethroids are active ingredients of long-lasting insecticidal nets, and of these, alpha-cypermethrin is the active ingredient of Interceptor® nets, which were mass distributed in 2012 and 2014 for *L. longipalpis* control in the periurban area of the city of Neiva, Department of Huila, Colombia (Secretaría de Salud de Neiva, unpublished data) because of a recent outbreak of visceral leishmaniasis (Gómez-Romero and Zambrano, 2012).

MATERIALS AND METHODS

For the bioassays, were used *L. longipalpis* females from the first filial generation of the wild sandflies collected with CDC light traps in the rural area of El Callejón village, municipality of Ricaurte, Cundinamarca, in the Magdalena River basin, Colombia. The specimens were breeding and maintained according to the procedures described by Modi and Tesh, (1983) at the insectary of the Entomology Group (Instituto Nacional de Salud). In the *L. longipalpis* area of origin, although there is not a regular application of insecticides for vector control made by municipal health authorities, human population apply eventually domestic insecticides for pest control and agricultural use, both with pyrethroids as the active ingredient.

The pyrethroids solutions were prepared in absolute alcohol Merck®. The compounds were acquired as neat grade material purchased from Chem Service® (West Chester, PA, USA): lambda-cyhalothrin (purity = 99.1 %), alpha-cypermethrin (purity = 99.5 %) and deltamethrin (purity = 99.3 %).

The concentration-mortality tests in *L. longipalpis* were conducted following the guidelines of the WHO, (1970) with two modifications: 1) Plastic tubes lined with impregnated papers at standard concentrations supplied by WHO, were replaced by 250 ml Wheaton glass bottles (with an internal area, including the lid, of 0.0341 m^2) treated with 1 ml of insecticide solution in absolute alcohol according to the protocol of the Centers for Disease Control and Prevention, CDC (Brogdon and McAllister, 1998); and 2) WHO kit holding tubes were replaced by observation recipients with plaster moistened especially for the maintenance of *Lutzomyia* spp. (Santamaría *et al.*, 2002). This observation recipient was used just once and then was discarded.

Groups with ten *L. longipalpis* females without access to a blood meal, from 1 to 3 days old, that was previously supplied with water and 30 % sugar solution *ad libitum* were exposed to different concentrations of the active ingredients of pyrethroids in the treated bottles.

After 1 h of exposure, the females were moved to the observation recipients with water, and saturated sugar

solutions supplied idem (embedded in cotton balls) and kept in polystyrene boxes. The mortality was recorded 24 h after exposure, monitoring the observation recipient under the stereomicroscope. A female fallen were considered dead if, after a soft touch with an entomological needle, it did not move (Marceló *et al*., 2014, Denlinger *et al*., 2015) or if it responded with some type of movement, but was not able to fly (WHO, 2013) and return to an upright position.

For each insecticide, different concentrations (between five and six) that caused between 0 % and 100 % mortality in the exposed groups of females were tested, using the median lethal concentrations of pyrethroids reported in other species of sandflies as a reference (Tetreault *et al.,* 2001; Álvarez *et al*., 2006). The concentrations determined were used for subsequent tests. Each set of tests included seven bottles with two different concentrations of each pyrethroid and a bottle treated just with the solvent (control). The sets of tests were conducted on different days. Each concentration was repeated between five and six times.

Each treated bottle was used a maximum of three times. Between tests, the bottles remained capped, covered with foil and refrigerated at 4 °C. During the tests, the temperature ranged between 23 and 25 °C, and the relative humidity was between 60 and 70 %. A probit analysis (concentration - mortality regression) was performed using the Finney method with the BioStat v.5 (2009) program.

RESULTS

In bioassays with concentrations defined for each insecticide, between five and six repetitions per concentration, a total of 1,090 *L. longipalpis* females were used. The mortality in the control bottles was low: 1.2 % (3 of 252 exposed females). Table 1 shows for each pyrethroid, the concentrations (µg/ml) that caused between 0 % and 100 % mortality in *L. longipalpis*. Table 2 shows the results of the probit analysis with the lethal concentrations for each pyrethroid, with their 95 % confidence intervals for *L. longipalpis*.

Comparing the median lethal concentrations of the three pyrethroids, lambda-cyhalothrin showed the highest degree of toxicity in *L. longipalpis* (the lowest LC_{50}), followed by alpha-cypermethrin and deltamethrin. The LC_{50} of lambda-cyhalothrin was 10.6 times less than the LC_{50} found for deltamethrin and 4.8 times less than the LC_{50} of alpha-cypermethrin. The LC_{50} of alpha-cypermethrin was 2.2 times lower than the one of deltamethrin.

DISCUSSION

The three pyrethroids tested were toxic to *L. longipalpis* at very low concentrations. For *L. longipalpis*, there are only three studies about concentration-mortality, one was conducted for the deltamethrin pyrethroid, in which the median lethal concentration reported was 2.5 mg/m^2 (Falcao *et al*., 1988). In the second study, the LC_{50} for lambda-cyhalothrin and deltamethrin were 0.23 µg/bottle and 0.92 µg/bottle, respectively (Denlinger *et al*., 2015).

In the third study, the LC_{50} for alpha-cypermethrin was 0.78 mg/m^2 (Pessoa *et al*., 2015). However, a direct comparison with the results of this study cannot be made, because of the crucial differences between methodologies.

Table 1. Pyrethroids concentrations (µg /ml) causing mortalities between 0 and 100% in *Lutzomyia longipalpis*

Pyrethroid	Concentration	Mortality %	(min – max)
Lambda-cyhalothrin	1.0	100	(100)
	0.5	100	(100)
	0.26	74.0	(50.0 - 100)
	0.034	18.0	(5.0 - 60.0)
	0.0034	11.8	(0 - 20.0)
	0.0017	3.9	(0 - 20.0)
Alpha-cypermethrin	3.4	85.7	(60.0 - 100)
	2.6	94.0	(80.0 - 100)
	1.7	88.5	(54.5 - 100)
	0.34	27.8	(10.0 - 35.7)
	0.034	3.9	(0 - 10.0)
Deltamethrin	17.0	100	(100)
	3.4	100	(100)
	1.7	85.0	(50.0 - 100)
	0.17	32.0	(5.0 - 54.5)
	0.017	10.2	(0 - 30.0)

Table 2. Lethal concentrations (µg /ml) for three pyrethroids in *Lutzomyia longipalpis*

Lethal concentrations	lambda-cyhalothrin n[1] = 302	alpha-cypermethrin n = 279	deltamethrin n = 267
LC_{25} (95 % CI[2])	0.02 (0.01 - 0.03)	0.08 (0.04 - 0.12)	0.22 (0.015 - 0.51)
LC_{50}	0.05 (0.04 - 0.06)	0.24 (0.16 - 0.35)	0.53 (0.38 - 0.68)
LC_{75}	0.17 (0.10 - 0.24)	0.73 (0.51 - 1.10)	1.30 (1.15 - 1.45)
LC_{95}	0.86 (0.45 - 1.27)	3.62 (2.21 - 5.03)	4.72 (4.54 - 4.90)
LC_{99}	2.71 (1.90 - 3.52)	11.11 (5.85 - 16.37)	11.68 (6.23 – 17.13)

[1]Total number of *L. longipalpis* females exposed
[2]Confidence intervals

As concerns; in the first study, they used blood-fed females for the bioassays, in the second one, they used 1000 ml or 1892 ml glass bottles as test chambers also used *L. longipalpis* females and males in the same proportion; and in the third study the authors do not report any information about the sex of the exposed sandflies, or about the device where exposure was performed, in which there may be areas not treated with the insecticide such as WHO exposure tube.

The WHO recommended dosages for indoor residual treatment against mosquitoes (WHO, 2006) and sandflies (WHO, 2010) for these pyrethroids (between 20 and 30 mg active ingredient/m^2) as well as other control measures, are approximately 253.2, 60.6 and 58.8 times greater than the LC_{99} obtained in *L. longipalpis* for lambda-cyhalothrin, alpha-cypermethrin and deltamethrin, respectively (using an internal area of the bottle and its lid of 0.0341 m^2 as a reference, for the calculation of LC_{99} in mg/m^2). Importantly, the lethal concentrations obtained under experimental conditions cannot be compared directly with field application dosages, because a laboratory determination does not consider insecticide losses produced by drag, photolysis, the surface where the insecticide is applied, and the potential contact time of the insect with the treated surface (Lagunes-Tejeda *et al.,* 2009).

Because the recommended operating level concentrations for sandflies are extrapolated from vector control of malaria, it would be advisable to study the effect of the pyrethroids at both, the recommended dosages and the LC found in this study on *L. longipalpis* because such high doses could have distinct results to those expected. For example, instead of observing an impact on mortality, high contact irritancy, may produce that the insect moves away before acquiring the lethal dose.

In another vector, knowledge of effects that cause lethal and sub-lethal concentrations of pyrethroids in the vector has been applied. Recently, a successful strategy was evaluated in *Aedes aegypti* resting sites within the home, they were focally-treated using the minimum effective concentration of the pyrethroid in the vector (<LD_{90}), which was equivalent to half of the WHO-recommended field application dosages (Manda *et al.,* 2013), which showed a greater efficacy in the measure, with less amount of insecticide.

Another context, in which the intervals of the toxic response to pyrethroids are useful, is in the comparison of the susceptibility between two populations of the same species. This data, especially the LC_{50} could be used in the comparison of the susceptibility of the population of *L. longipalpis* from the Magdalena River basin, with 1) other populations of *L. longipalpis* subjected to continuous pressure with different chemical control measures, 2) the same population in a longitudinal study to track changes over time in response to pyrethroids after an eventual intervention. The comparison can be made through the calculation of the resistance ratio of 50 % (RR_{50}) (Mazzarri *et al.,*1997; Pessoa *et al.,* 2015).

In *L. longipalpis,* indicators of susceptibility to lambda-cyhalothrin, deltamethrin y alpha-cypermethrin have been estimated before, either with the method used by the Centers for Disease Control and Prevention (Marceló *et al*., 2014), or by establishing lethal times at fixed concentrations of insecticides (Mazzari *et al.,* 1997; Alexander *et al.,* 2009) or lethal concentrations at fixed times, according to the method proposed by WHO (Pessoa *et al.,* 2015) or with significant modifications (Denlinger *et al*., 2015). The method used to estimate lethal concentrations in this study was the one reported by the WHO, but the standard impregnated papers were not used. These papers have disadvantages, such as the high cost of shipping and short expiration. Further, in the WHO device for insecticide exposure, the top and bottom bases of the cylinder are not covered by impregnated paper, so the insects may take different insecticide amounts in each replicate, which can lead to errors in the results. The glass bottle treated according to CDC guidelines is more versatile, it can be treated at any concentration with absolute alcohol and technical grade insecticides, and the entire surface available for insect exposure is treated.

CONCLUSION

In conclusion, through the traditional method of WHO, but using the glass bottle as an exposure chamber, concentrations of three pyrethroids that kill 25, 50, 75, 95

and 99 % of females exposed were established for *L. longipalpis*, which comes from an area with sporadic application of insecticides (domestic or agricultural use). These data could be mainly useful in studies on the effects of sub-lethal concentrations of these pyrethroids in the target population and other *L. longipalpis* populations from Magdalena river valley in Colombia, because of its genetic similarities (Hoyos *et al.,* 2012).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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