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

Erika SANTAMARIA1*, Catalina MARCELÓ1

1Grupo de Entomología, Instituto Nacional de Salud, Calle 26 n°. 51-20, Bogotá, Colombia.

*For correspondence: esantamaria@ins.gov.co

Received: 29th August 2018, Returned for revision: 1st October 2018, Accepted: 7th February 2019.

Citation/Citar este artículo como: Santamaría E, Marceló C. Toxic activity of pyrethroids in *Lutzomyia longipalpis* (Diptera: Psychodidae) from Magdalena River basin, Colombia. Acta biol. Colomb. 2019;24(2):391-396. DOI: http://dx.doi.org/10.15446/abc.v24n2.74570

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 lambdacaloftrina, deltamatrina 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 utilizó 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 piretroido, 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 % (LC$_{50}$ y LC$_{95}$) de las hembras expuestas de *L. longipalpis* fueron de 0.05 y 0.86 para la lambdacaloftrina, 0.24 y 3.62 para la alfacipermetrina y 0.53 y 4.72 para la deltamatrina. Basados en las LC$_{50}$ obtenidas, la lambdacaloftrina fue el piretroido con mayor toxicidad para *L. longipalpis*, seguido por la alfacipermetrina y la deltamatrina. 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 (LC50) and 95 (LC95), 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 LC50 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 Modí 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 m²) treated with 1 ml of insecticide solution in absolute alcohol according to the protocol of the Centers for Disease Control and Prevention, CDC (Brdgon and McAllister, 1998); and 2) WHO kit holding tubes were replaced by observation recipients with plaster moistened especially for the maintenance of Lutzomyia spp. (Santamaria 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
Toxic activity of pyrethroids in *L. longipalpis*

Acta biol. Colomb., 24(2):391-396, Mayo - Agosto 2019

- 393

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$^2$ (Falcão *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)         |
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/m²) as well as other control measures, are approximately 253.2, 60.6 and 58.8 times greater than the LC₉₀ obtained in L. longipalpis for lambda-cyhalothrin, alphacypermethrin and deltamethrin, respectively (using an internal area of the bottle and its lid of 0.0341 m² as a reference, for the calculation of LC₉₀ in mg/m²). 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 (<LD₂₀), 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 LC₉₀ 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 % (RR₉₀) (Mazzari 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 (Marcelo 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).

**ACKNOWLEDGMENT**

The work was supported by Instituto Nacional de Salud and Colciencias (research project 124349326165, young research program, 2013 and doctorate program “Generación del Bicentenario,” 2010). The authors are grateful with Marco Fidel Suarez from the Entomology Group (Instituto Nacional de Salud) for his valuable assistance in rearing *L. longipalpis* sandflies.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**REFERENCES**

Alexander B, Barros VC, De Souza SF, Barros SS, Teodoró LP, Soares ZR. Susceptibility to chemical insecticides of two Brazilian populations of the visceral leishmaniasis vector *Lutzomyia longipalpis* (Diptera: Psychodidae). Trop Med Int Health. 2009;14(10):1272-1277. Doi: http://dx.doi.org/10.1111/j.1365-3156.2009.02371.x.

Álvarez L, Duran Y, González A, Suárez J, Oviedo M. Concentraciones letales (CL₉₀ y CL₅₀) y dosis diagnósticas de fenitrotión y lambdacioldotrina para *Lutzomyia evansi* (Diptera: Psychodidae) de los Pajones, estado Trujillo, Venezuela. Bol Mal Salud Amb. 2006;46(1):31-37.

Barata RA, Michalsky EM, Fujiwara RT, Franca-Silva JC, Rocha MF, Dias ES. Assessment of sandfly (Diptera, Psychodidae) control using cypermethrin in an endemic area for visceral leishmaniasis, Montes Claros, Minas Gerais State, Brazil. Cad Saude Publica. 2011;27(11):2117-2123. Doi: http://dx.doi.org/10.1590/S0102-311X2011001100005.

Bray DP, Álvez GB, Dorval ME, Brazil RP, Hamilton JGC. Synthetic sex pheromone attracts the leishmaniasis vector *Lutzomyia longipalpis* to experimental chicken sheds treated with insecticide. Parasit Vectors. 2010;3:16. Doi: http://dx.doi.org/10.1186/1756-3305-3-16.

Brogdon WG, McAllister JC. Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. J Am Mosq Control Assoc. 1998;14(2):159-164.

Courteney O, Gillingwater K, Gomes PA, Garcez LM, Davies CR. Deltamethrin impregnated bednets reduce human landing rates of sandfly vector *Lutzomyia longipalpis* in Amazon households. Med Vet Entomol. 2007;21(2):168-176. Doi: http://dx.doi.org/10.1111/j.1365-2915.2007.00678.x

David JR, Stamm LM, Bezerra HS, Souza RN, Killick-Kendrick R, Lima JW. Deltamethrin impregnated dog collars have a potent anti-feeding and insecticidal effect on *Lutzomyia longipalpis* and *Lutzomyia migonei*. Mem Inst Oswaldo Cruz. 2001;96(6):839-847.

Denlinger DS, Lozano-Fuentes S, Lawyer PG, Black WC, Bernhardt SA. Assessing insecticide susceptibility of laboratory *Lutzomyia longipalpis* and *Phlebotomus papatasi* sand flies (Diptera: Psychodidae: Phlebotominae). J Med Entomol. 2015;52(5):1003-1012 Doi: http://dx.doi.org/http://10.1093/jme/tjv091.

Falcao AR, Pinto CT, Gontijo CM. Susceptibility of *Lutzomyia longipalpis* to deltamethrin. Mem Inst Oswaldo Cruz. 1988;83(3):395-96.

Feliciangeli MD, Mazarri MB, Blas SS, Zerpa O. Control trial of *Lutzomyia longipalpis* s.l. in the Island of Margarita, Venezuela. Trop Med Int Health. 2003;8(12):1131-36.

Gómez-Romero SE, Zambrano P. Informe del evento leishmaniasis hasta el XIII periodo epidemiológico del año 2012. [Internet].Instituto Nacional de Salud, Bogotá, D.C., Colombia. 2012. Disponible en: http://www.ins.gov.co/lineas-de-accion/SubdirecccionVigilancia/Informe%20de%20Evento%20Epidemiologico/Forms/AllItems.aspx. Cited: 14 June 2014.

Hoyos R, Uribe S, Vélez ID. Tipificación de especímenes colombianos de *Lutzomyia longipalpis* (Diptera: Psychodidae) mediante “Código de Barras”. Rev Colomb Entomol. 2012;38(1):134-140.

Hubert JJ. Bioassay. USA: Kendall Hunt Pub. Co.; 1980. 164 p.

Kelly D, Mustafa Z, Dye C. Differential application of lambdacyhalothrin to control the sandfly *Lutzomyia longipalpis*. Med Vet Entomol. 1997;11(1):13-24.

Lagunes-Tejeda A, Rodríguez-Maciel C, De Loera-Barocio JC. Susceptibilidad a insecticidas en poblaciones de artrópodos de México. *Agrociencia*. 2009;43(2):173-196.

Manda H, Shah P, Polsomboon S, Chareonviriyaphap T, Castro-Llanos F, Morrison A, et al. Contact irritant responses of *Aedes aegypti* using sublethal concentration and focal application of pyrethroid chemicals. PLoS Negl Trop Dis. 2013;7(2):e2074. Doi: http://dx.doi.org/10.1371/journal.pntd.0002074.

Marceló C, Cabrera OL, Santamaría E. Indicadores de sensibilidad de una cepa experimental de *Lutzomyia longipalpis* (Diptera: Psychodidae) a tres insecticidas de uso en salud pública en Colombia. Biomedica. 2014;34(4):624-30. Doi: https://doi.org/10.7705/biomedica.v34i4.2233.

Mazarri MB, Feliciangeli MD, Maroli M, Hernandez A, Bravo A. Susceptibility of *Lutzomyia longipalpis* (Diptera: Psychodidae) to selected insecticides in an endemic focus of visceral leishmaniasis in Venezuela. J Am Mosq Control Assoc. 1997;13(4):335-341.
Ministerio de la Protección Social, Instituto Nacional de Salud, Organización Panamericana de la Salud. Gestión para la vigilancia entomológica y control de la transmisión de leishmaniasis. [Internet]. Guía Técnica, Colombia, [actualización 21 noviembre 2013]. 2012. Disponible en: http://www.ins.gov.co/temas-de-interes/Paginas/leishmaniasis-visceral.aspx

Ministerio da Saúde. Fundação Nacional de Saúde. Manual de vigilância e controle da Leishmaniose Visceral, 1a ed. Ministério da Saúde. Brasília: Editora do Ministério da Saúde; 2013. 120 p.

Modi GB, Tesh RB. A simple technique for mass rearing Lutzomyia longipalpis and Phlebotomus papatasi (Diptera: Psychodidae) in the laboratory. J Med Entomol. 1983;20(5):568-569

Molyneux DH. Control. In Cox FEG, editor (s). Modern parasitology: a textbook of parasitology. United Kingdom: Blackwell Science, Cambridge; 1993. p. 243-63.

Pessoa GC, Lopes JV, Rocha MF, Pinheiro LC, Rosa AC, Michalsky EM, et al. Baseline susceptibility to alphacypermethrin in Lutzomyia longipalpis (Lutz & Neiva, 1912) from Lapinha Cave (Brazil). ParasitVectors. 2015;8(1):469. Doi: http://doi.org/10.1186/s13071-015-1076-y.

Romero GA, Boelaert M. Control of visceral leishmaniasis in Latin America - a systematic review. PLoS Negl Trop Dis. 2010;4(1):e584. Doi: http://10.1371/journal.pntd.0000584.

Santamaría E, Munstermann LE, Ferro C. Estandarización del método propuesto por la Organización Mundial de la Salud para determinar los niveles de susceptibilidad de los vectores de leishmaniasis a insecticidas. Biomedica. 2002;22(2):211-218. Doi: https://doi.org/10.7705/biomedica.v22i2.1146

Tetreault GE, Zayed AE, Hanafi HA, Beavers GM, Zeichner BC. Susceptibility of sandflies to selected insecticides in North Africa and the Middles East. J Am Mosq Control Assoc. 2001;17(1):23-27.

World Expert Committee on Insecticides and World Health Organization. Insecticide resistance and vector control: WHO technical report series N. 443. Geneva; 1970. 279 p. Available in: http://www.who.int/iris/handle/10665/40771

World Health Organization. Pesticides and their application. Department of control of neglected tropical diseases WHO Pesticide Evaluation Scheme (WHOPES), 6th ed. Geneva; 2006. 114 p. Available in: http://www.who.int/whopes/resources/who_cds_ntd_whopes_gcdpp_2006.1/en/

World Health Organization. Control of the leishmaniasis. WHO technical report series N. 949. Geneva; 2010. 186 p. Available in: http://www.who.int/neglected_diseases/resources/who_trs_949/en/

World Health Organization. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva; 2013. 48 p. Available in: http://apps.who.int/iris/bitstream/handle/10665/250677/9789241511575-eng.pdf?sequence=1