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Larval Susceptibility of Two *Culex quinquefasciatus* Populations (Diptera: Culicidae) Temephos® in the City of Naviraí, MS, Brazil

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Abstract: The control mosquito populations in Brazil is needed to prevent disease transmission and nuisance to man, and avoid causing deaths and economic losses. The susceptibility of two populations of *Culex quinquefasciatus* to the larvicide temephos was evaluated. Larvae were collected in septic tanks at the neighborhoods of Vila Nova and Varjão (Naviraí, Mato Grosso do Sul, Brazil), and submitted to different concentrations of the insecticide, being possible to calculate lethal concentrations. The results showed that there is resistance to this organophosphate, when considering the degree of mortality at the diagnosis concentration (DC), indicated by WHO, what was confirmed by the high values found for LCs. We discussed the role of larvicide in national dengue control program and the agricultural use of organophosphates in the region. The results indicate the need to adopt integrated management practices vectors against this mosquito that causes discomfort.

Keywords: insecticide; chemical control; organophosphate; mosquito; resistance

1. INTRODUCTION

According to the Food and Agriculture Organization of the United Nations (FAO), Brazil has been one of the largest consumers of insecticides using 1.15 ton per 1000 ha, leaving behind a few countries like the United States, Portugal and Costa Rica (2.31, 6.55 and 21.84 ton per 1000 ha, respectively), being agriculture the main responsible for it [1]. It is clear that the intensive use of these compounds causes problems due to their nonselective action, eliminating non-target organisms and causing environmental impacts [2]. The continuous and indiscriminate use of insecticides in agriculture also causes the selection of resistant populations around the world and this leads to a constant exchange of products, reducing the effectiveness of this plague control method and encouraging the search for new insecticides [3-5].

Temephos is a synthetic product from the organophosphates group, which acts on the central and peripheral nervous system by inhibiting acetylcholinesterase, an enzyme that mediates nerve impulses. It's effective as larvicide for mosquitoes, it is inexpensive and it has low toxicity to mammals and, for this reason, it's widely used in mosquito control efforts [6, 7].

Mosquitoes are responsible for transmission of pathogens that cause diseases such as dengue, malaria, elephantiasis, West Nile and St. Louis encephalitis, among others, and are still an important nuisance factor to human populations [8, 9]. The mosquito *Culex quinquefasciatus* (Say) has marked

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anthropophilic habit and is widely distributed, living in almost all urban areas, especially the ones without adequate basic infrastructure [10], proliferating in wastewater rich in organic matter and waste of human activities. Thus, exogenous factors such as climate change, urban sprawl, lack of health infrastructure, lack of guidance and health education, environmental imbalance and the availability of food and breeding are related to population increase of mosquito's larvae [9]. In addition to diseases that they can transmit, mosquitoes bites cause allergic reactions leading to more severe hypersensitivity, especially in children and in the elderly [11, 12]. The intensity of the attacks irritates and causes sleepless nights, stress, reduction in quality of life and decreased productivity, serious consequences in human populations [13].

Strategies for population control of *C. quinquefasciatus* may be characterized simply as biological, mechanical and/or chemical control. Recently, to obtain better results, it has been adopted the principles of Integrated Pest Management (IPM), which is a combination of all possible methods, including legislation support, community participation and environmental education [14]. IPM has been used successfully in Brazil. However, chemical control directed to adult mosquitoes or their immature forms has often been used as the only strategy, because it's erroneously considered faster and more efficient. In these cases, organophosphates, chlorophosphates and pyrethroids are the most used insecticides [12, 15].

Besides the development of resistance to insecticides that are being used in mosquito control, there is still the possibility for these plagues to acquire cross-resistance to products used in agriculture, and before a particular product is elected to be used, it's important to check the susceptibility of local populations [9]. Cross-resistance can occur in response to the intensive use of pesticides, or due to the migration of individuals from resistant populations [12, 16-19].

In Brazil, literature records resistance of *C. quinquefasciatus* to organophosphates in Rio Grande do Sul [20] and São Paulo [21-23], resulting in operational implications, such as changing the frequency of insecticide applications, equipment replacement, continuity in control programs, adjustment of evaluation and monitoring methods and, in consequence, financial implications. Thus, it has been recommend by the World Health Organization (WHO) and local agencies the adoption of standardized tests [17, 24] in order to establish the resistance status of the population.

The city of Naviraí has an area of 3,194 km^2 with a population of approximately 46,000 inhabitants and is the seventh most populous city in the state of Mato Grosso do Sul, Brazil. It is located in the Atlantic Forest biome and its main economic activities are agriculture, industry and trade [25]. The city counts on a program for monitoring and controling *Aedes aegypti* that is currently performed by Municipal Vector Control Center in partnership with the Municipal Health Surveillance. For other mosquitoes or insect vectors there are no control programs. The present study aimed to evaluate the susceptibility of *C. quinquefasciatus* larvae to the insecticide temephos in the municipality of Naviraí, in order to provide parameters for management programs.

2. MATERIAL AND METHODS

Initially, a survey was conducted, using data from the Municipal Center for Vector Control, in order to identify neighborhoods with higher incidence of complaints about the nuisance caused by mosquito house *C. quinquefasciatus*. After that, residents were interviewed in order to verify the importance of nuisance caused by mosquitoes and can demand control.

The susceptibility bioassays were performed using a method proposed by WHO [17, 24]. Immature *C. quinquefasciatus* were collected from septic tanks in Varjão (January-February 2013) and Vila Nova (April-May 2013) quarters. Pupae and 4th stage were discarded and the 1st and 2nd stage larvae were kept in plastic containers and fed with an aqueous suspension of fish feed at 0.1% (w/v) until reaching the 3rd stage of development when they were used for bioassays. Temephos used in the study was the commercial product Fersol 500 (Fersol, Brazil) with 50% of active ingredient in an emulsifiable concentrate formulation. The lethal concentrations to 50%, 90% and 99% of individuals (CL_{50}, CL_{90} and CL_{99}) were calculated. An insect population is considered to be susceptible when the mortality rate is above 98%; there is suspicion of resistance between 80% and 97% of death; and the population is considered resistant when mortality rate is below 80%. These settings are determined when using a concentration diagnosis in a population of insect field [26, 27].

Diluted solutions were prepared from a more concentrated one (1 ppm), using unchlorinated water, resulting in 1,000 mL of each final concentration. After that, solutions were distributed into four 250 mL plastic vials and groups of 25 larvae in the 3rd stage were transferred to them and fed with macerated fish feed. All assays were performed in four replicates at room temperature (27 °C ± 2 °C). Temephos and water were used as positive and negative control, respectively.

Larval mortality was observed after 24 hours of exposure to the test solutions. Those who had no spontaneous movement and did not respond to physical stimulus were considered dead. Mortality rates were subjected to probit analysis, using the program POLO- PC [28, 29].

3. RESULTS AND DISCUSSION

From the analysis of data from the Municipal Center for Vector Control, it was confirmed that two districts (Vila Nova and Varjão) have a high rate of complaints and demands for control of *C. quinquefasciatus*. In the district Vila Nova, about 80% of people have complained that the mosquito bothers overnight and Varjão district, about half the people cited the same problem. It was observed that both districts have no sanitation and there are loads of open septic tanks or badly closed. Industrial wastewater treatment tanks occur near the district Vila Nova. These situations favor the proliferation of the mosquito *C. quinquefasciatus*. Through the elimination of these environmental factors should be the first approach to a good management program.

The insecticide concentrations used in this study were 0.001, 0.004, 0.016 and 0.064 ppm. The average percentages of mortality are shown in **Table 1**. It is observed that there was a variation between 0 and 100% mortality at the concentrations used in this bioassay.

Table 1. Percentage of mortality (mean ± standard deviation) for *C. quinquefasciatus* larvae populations of two neighborhoods in Naviraí, MS, subjected to different concentrations of Temephos.

	Mortality (% ± dp)	
Concentration (ppm)	Vila Nova	Varjão
0.001	0	0
0.004	18 ± 2.23	20 ± 3.26
0.016	98 ± 2.00	97 ± 2.00
0.064	100	100

According to Campos (2011) [15] to susceptible populations of *C. quinquefasciatus* to temephos, the LC_{50} and LC_{95} values are 0.0011 ppm and 0.003 ppm, respectively, however these values may vary in each region. According to WHO [17] the concentration diagnostic (CD) for this species of mosquito to temephos is 0.002 ppm. Therefore, the lowest concentration (0,001 ppm) used in this work should cause near 50% of mortality and the concentration 0.004 ppm, two times greater than the CD adopted by WHO [17], should certainly cause total mortality in larvae. Working up with a mosquito colony likely, the CD is determined to larvae for various tests as the lowest concentration with ability to kill all individuals in 24 hours or causing 99.9% mortality ($LC_{99,9}$) in testing multiple concentrations [35, 36]. These results indicate that the population of Varjão and Vila Nova are resistant to temephos.

Resistance is confirmed by the LC_{50} and LC_{99} values obtained in this study as 0.005 ppm (0.004 – 0.006) and 0.02 (0.0015 – 0.02), respectively (**Table 2**). Therefore, LC_{50} of this study is 5 times higher than that of Campos (2011) [15] and the LC_{99} is 10 times higher than that of WHO [17].

Table 2. Lethal Concentrations (limits) (ppm) for two populations of *C. quinquefasciatus* from Naviraí, MS, to Temephos.

Local	**LC_{50}**	**LC_{90}**	**LC_{99}**
Vila Nova	0.005 (0.004 – 0.006)	0.011 (0.009 – 0.014)	0.019(0.015 – 0.019)
Varjão	0.006 (0,005 – 0,007)	0.012 (0,010 – 0,015)	0.020 (0.016 – 0.029)

The resistance observed in the city of Naviraí to the studied insecticide may be related to the use of agricultural products in the region, as pointed out in other works [30]. According Scudeler et al. [31], the continued use of temephos for years in this city led to the development of resistance in *A. aegypti* populations. It is observed that the use of temephos, provided by the federal government for the *A. aegypti* control in the city. Therefore, it could be possible that the indiscriminate use of this organophosphate is even affecting the resistance profile in *C. quinquefasciatus* populations. As the breeding occur mainly in residential septic tanks, the use of products for the controlling dengue fever in water tanks of households could be considered the main source of continuous selection of resistant populations of *C. quinquefasciatus*, into the septic tanks.

Temephos is a low cost organophosphate that has proven its efficiency since the 70s. It has been used in Brazil for more than thirty years as a larvicide, as vector control for dengue fever. Resistance in *A. aegypti* and *C. quinquefasciatus* to temephos is, however, occurring in several regions of the country [23, 32, 33] and for this compound continue to be used as a vector control, it is mandatory to monitor resistant populations [9, 21, 34].

4. CONCLUSION

The populations of *C. quinquefasciatus* of Naviraí and Varjão, MS, are resistant to temephos. There is an urgent need to replace the insecticide temephos for continued control of *C. quinquefasciatus*.

5. REFERENCES AND NOTES

[1] Available from: http://faostat.fao.org/Site/679/DesktopDefault.aspx?PageID=679#ancor. Access March 2014.

[2] Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Diretrizes nacionais para a prevenção e controle de epidemias de dengue. (Série A. Normas e Manuais Técnicos), Brasília: Ministério da Saúde, 2009, 160 p.

[3] Forattini, O. P. *Rev. Saude Publica* **1998**, *32*, 497. [CrossRef]

[4] Chalegre, K. D. M. Diagnóstico da resistência do vetor *Culex quinquefasciatus* ao biolarvicida *Bacillus sphaericus*. [Master´s thesis] Recife, Brazil: Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, 2008.

[5] Available from: http://www.irac-online.org/documents/public-health-irm-poster/?ext=pdf. Access May, 2014.

[6] Gambarra, W. P. T. Tecnologias de georreferenciamento e genética molecular aplicados à avaliação da resistência de *Aedes (Stegomyia) aegypti* (L.) (Diptera: Culicidae) ao Temephos. [Master´s thesis] Campina Grande, Brazil: Centro de Ciências e Tecnologias, Universidade Estadual de Paraíba, 2010.

[7] Available from: http://www.encoppragas.com.br/inseticidas_92.html. Access November 2012.

[8] Peruzin, M. C. J. Análises comparativas populacionais de *Culex quinquefaciatus* de dois locais do Estado de São Paulo. [Master´s thesis] São Paulo, Brazil: Instituto de Ciências Biomédicas da Universidade de São Paulo, 2009

[9] Brasil. Guia de vigilância do Culex quinquefasciatus. Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. Série A. Normas e Manuais Técnicos. 1ª Edição. Brasília - DF. Araújo, F.A.A. & M. Santalucia, Coord., 2011, 76 p.

[10] Available from: http://www.ib.unicamp.br/profs/eco_aplicada/artigos_tecnicos.htm. Access September 2011.

[11] Ruas-Neto, A.; Silveira, S. M. *Mem. Inst. O. Cruz* **1989**, *84*, 39. [CrossRef]

[12] Alves, S. N.; Tiburcio, J. D.; Melo, A. L. *Rev. Soc. Bras. Med. Trop.* **2011**, *44*, 486. [CrossRef]

[13] Forattini, O. P.; Massad, E. *Ecosyst. Health*. **1998**, *4*, 9. [CrossRef]

[14] Available from: http://bvsms.saude.gov.br/bvs/publicacoes/funasa/controle_vetores.pdf. Access April 2015

[15] Campos, J. Análise Citológica de populações de *Aedes aegypti* (Linnaeus, 1762) e *Culex quinquefasciatus* Say, 1823 (Diptera, Culicinae). [Doctoral dissertation] Instituto de Biologia, Universidade Estadual de Campinas, 2011.

[16] Andrade, C. F. S.; Castello Branco Jr., A. *Rev. Saude Publica* **1991**, *25*, 367.

[17] Available from: http://apps.who.int/iris/handle/10665/37432#sthash.zOlrtpbM.dpuf. Access April 2015.

[18] Adler, P. H.; Cheke, R. A.; Post, R. *J. Infec. Gen. Evol*. **2010**, *10*, 846. [CrossRef]

[19] Teixeira, T. Z.; Arruda, E. J.; Andrade, C. F. S.; Crispim, B. A.; Fernandes, M. F.; Silva, E. S.; Nakamura, A. K. S. *BioAssay* **2014**, *9*, 1. [CrossRef]

[20] Ruas-Neto, A. L.; Silveira S. M., Colares E. R. C. *Cad. Saude Publica*. **1994**, *10*, 222. [CrossRef]

[21] Bracco, J. E.; Dalbon, M.; Marinotti, O.; Barata, J. M. S. *Rev. Saude Publica* **1997**, *31*, 182. [CrossRef]

[22] Bracco J. E.; Barata, J. M. S.; Marinotti, O. *Mem. Inst. O. Cruz* **1999**, *94*, 115. [CrossRef]

[23] Campos, J.; Andrade, C. F. S. *Rev. Saude Publica* **2003**, *37*, 523. [CrossRef]

[24] WHO - World Health Organization. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO-VBC 1981.

[25] Avalilable from: http://www.ibge.gov.br/cidadesat/painel/painel.php?codmun=500570#. Access January 2013.

[26] Davidson, G.; Zahar, A. R. *Bull. World Health Organ*. **1973**, *49*, 475.

[27] WHO - World Health Organization. Test procedures for insecticide resistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces. Report of an informal consultation, WHO/CDS/CPC/MAL/98.12.

[28] Finney D. Probit Analysis: a statistical treatment of the sigmoid response curve. University Press, Cambridge, 1974, p.50-55.

[29] LeOra Software. Polo-PC, probit or logit analysis.Berkeley (CA), 1987.

[30] Leong, C. S.; Chen, C. D.; Lee, H. L.; Izzul, A. A.; Chia, K. H. M.; Low, V. L.; Lau, K.W.; Sofian-Azirun, M. *Med. & Health* **2011**, *6*, 270.

[31] Scudeler, C. G. S. Silva, E. L. Teixeira, T. Z. Arruda, E. J. Fernandes, M. F. Cabrini, I. Andrade, C. F. S. 2011. Avaliação da susceptibilidade larval de *Aedes aegypti* em Naviraí – MS ao inseticida organofosforado Temephos. Anais DIERN. v. 1 pg.

[32] Braga, I. A.; Lima, J. B. P.; Soares, S. S.; Valle, D. *Mem. Inst. O. Cruz* **2004**, *99*, 199. [CrossRef]

[33] Macoris, M. L. G.; Andrighetti, M. T. M.; Nalon, K. C. R.; Garbeloto, V. C.; Caldas-Júnior, A. L. *Dengue Bull.* **2005**, *29*, 176.

[34] Barreto, C. F. *Rev. Elet. Fac. Mon. Bel.* **2005**, *1*, 62.

[35] Campos, J.; Andrade, C. F. S. *Rev. Saude Publica* **2001,** *35*, 232. [CrossRef]

[36] Brown, A. W .A. *J. Am. Mosq. Control Assoc*. **1986**, *2*, 123.